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California Affordable Housing Policies Fail in Wealthy Towns: A Case Study of the CityWalk Project

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Abstract

To remedy its lack of affordable housing, the state of California has passed legislation aimed at implementing fair housing principles, including rules that require the creation of low-income housing in cities throughout the state. In San Ramon, a new development plan has been approved which will create thousands of housing units, retail developments, parks, a new hotel, and parking amenities. Called CityWalk, the new development projects a vision of a "walkable city," one in which people can live and work in the same district, never having to commute by car again. The reality, however, is that San Ramon's housing prices are likely inaccessible for the workers that CityWalk is hoping to attract -- the retail salespeople, restaurant cooks and servers, hotel maids and janitors, among other low-wage employees, who might actually work in the immediate vicinity. Several methods were used to determine whether or not CityWalk will meet the needs of the workers who need housing, gauge the level and possible ramifications of community and stakeholder input into the project, and shed light on the broader question of whether affordability thresholds based on relatively high percentages of area median income make sense in the context of highly polarized, post-industrial economies. American Community Survey data from 2020 was analyzed to profile the incomes, existing housing characteristics, and commute times of both the current residents of San Ramon and the workers who currently commute to the area. That data was juxtaposed with economic data from the state of California describing the median wages in the area for people holding the jobs CityWalk hopes to create. Finally, meeting minutes from San Ramon's planning meetings were analyzed to measure the degree of stakeholder involvement in the planning process. Approximately 0% of the San Ramon workers eligible for the affordable housing units at all three levels could afford those units when affordability was defined according to the federal definition of 'rent burden' (no more than 30% of income spent on rent). Opening the aperture to include workers who might improve their rent burden (by paying less than the Bay area average of 44% of income on rent) while still devoting high proportions of their income to rent still yielded very small slices of the worker population who might benefit: approximately 12%, 16%, and 14% of the work force, depending on the affordability level. Examination of the 351 relevant meetings held by San Ramon from 2019 through 2022 yielded only 44 mentions of the CityWalk project, for an average of one mention every eight meetings. Throughout the process, no more than 103 citizens participated, or less than one person, on average, every three meetings. This study concludes that the affordable housing allocation in the CityWalk project will serve neither the workers who will staff the central business district nor the current residents of San Ramon, a pattern likely to limit the utility of California's affordable housing rules in any wealthy enclave. Furthermore, San Ramon's planning process did not successfully gather input from either city residents or commuters the city sought to target. This research accordingly suggests policy makers and citizens alike may need to think deeper about both the execution and impacts of affordable housing in wealthy California towns.

Keywords: City planning, Affordable housing, Housing policy, Bay rea housing, Housing and urban development, Regional housing needs allocation (RHNA)

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

1. Introduction

California's state government has called for the creation of 2.5 million new housing units (among them 643,352 very low-income (30% AMI) and 384,910 low-income (50% AMI) units) to relieve severe shortfalls in housing supply and lower the cost of housing for California (State of California, 2021). As per California state requirements, 15% of these units are designated "affordable," but the high Area Median Income (AMI) of a city like San Ramon means that even apartments set at the lowest threshold (50% of AMI) might be too expensive for people who work in the low-income service sector catering to the needs of the high-income population. This bifurcated socio-economic pattern is described as the "global city" (Sassen, 1991) or "dual city" (Castells, 1989) and has been studied in the scholarly literature by Hamnett (1994).

San Ramon is currently embarking on an ambitious new development plan, CityWalk, that will include 4,500 new housing units on 138 acres of undeveloped land, as well as new retail development, open space and park land, a new hotel, and parking amenities (City of San Ramon, 2020a). CityWalk's goal of a 'walkable' city would require that the people who work in the city center-- many of whom are not the city's high-income residents-- should be the ones who live nearby, suggesting that the development should reflect, at least in part, the needs of the low-income central city workforce (City of San Ramon, 2020a). Currently, the housing in San Ramon largely prevents these large low-income service sector workers from living near where they work, and the high proportion of commuters to San Ramon incurs multiple costs in the form of commuting time, strained transportation infrastructure and traffic, and environmental consequences -- among other ramifications.

This paper aims to address the following questions: Have the needs of this largely-commuter workforce been factored into San Ramon's plans for CityWalk? Do the development's "affordable" homes fit the socioeconomic needs of San Ramon's low-income workers? How has San Ramon's planning process incorporated -- or failed to incorporate -- the input of these stakeholders who are potential but not current residents of the city, and might that be reflected in the nature of those plans?

2. Materials and Methods

We primarily relied on 2020 American Community Survey data on both San Ramon residents and respondents who listed Contra Costa County (the smallest unit of analysis available, and the county to which San Ramon belongs) as their place of employment in order to profile the resident and commuter workforce populations (US Census Bureau, 2020). Google sheets were used to collate, analyze, and graphically depict this data. Workers who reported that they were working at home (commute time = 0) were excluded from the data sets on commuters. For data on the development itself, we relied on published documents describing the plans for CityWalk and its new rental units. Data on typical wages for various occupations and labor market sectors in the area were obtained from the State of California's Department of Labor. Analysis of citizen participation in planning meetings was done by acquiring published meeting notes from the City of San Ramon's website.

3. Results

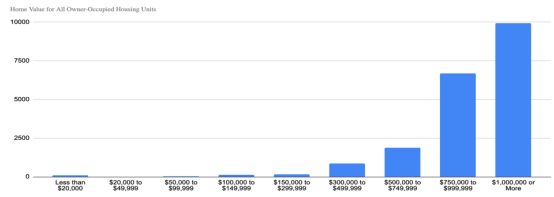

Figure 1. Home values in San Ramon

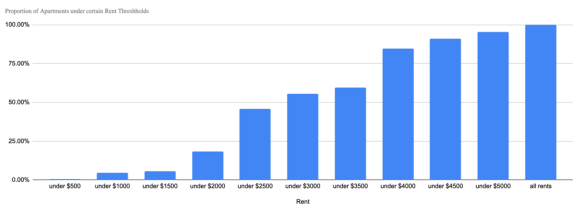

Figure 2. The cumulative distribution (or 'rent hill') of rents in San Ramon **(all figs -1)**

Journal of Research High School

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

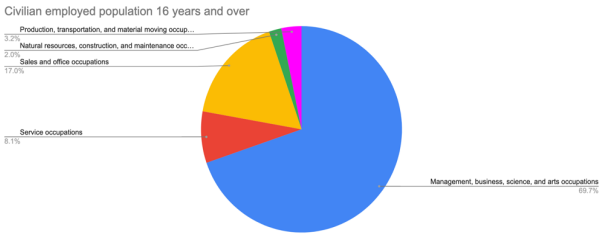

Figure 3. Occupations of San Ramon residents

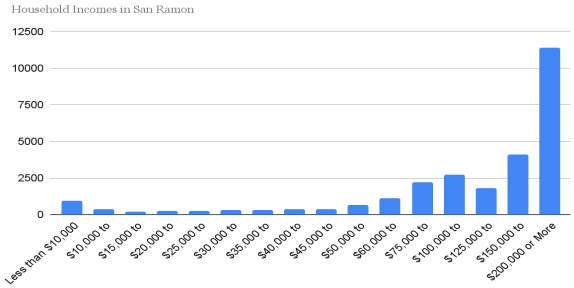

Figure 4. The income distribution of San Ramon residents

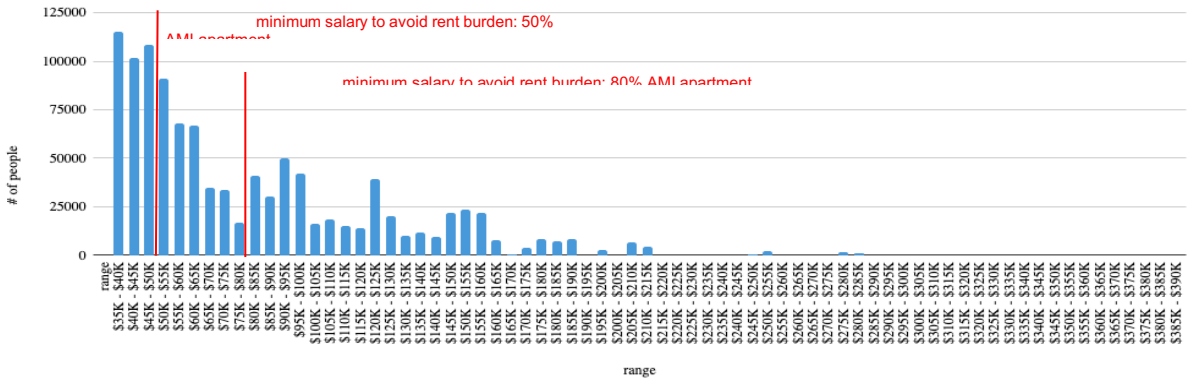

Figure 5. Distribution of salaries in Contra Costa county's labor market

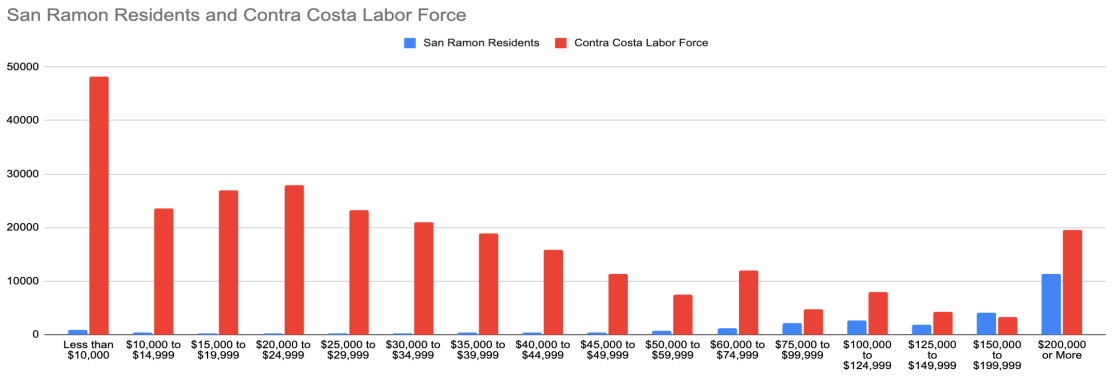

Figure 6. The distribution of household income versus labor market salaries in the San Ramon area

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

Table 1. Median salaries in Contra Costa county for Service sector/Central Business District jobs with at least 10,000 estimated employees

Occupational Title	May 2021 Employment Estimates	Mean Hourly Wage ($)	Mean Annual Wage ($)
Home Health and Personal Care Aides	47,070	$18.33	38,120.00
Cashiers	28,000	$18.48	38,443.00
Fast Food and Counter Workers	22,520	$18.63	38,751.00
Retail Salespersons	21,220	$20.68	43,018.00
Office Clerks, General	16,120	$25.19	52,387.00
Stockers and Order Fillers	15,110	$21.30	44,294.00
Janitors and Cleaners, Except Maids and Housekeeping Cleaners	12,910	$21.70	45,145.00
Customer Service Representatives	11,860	$24.73	51,431.00
Secretaries and Administrative Assistants, Except Legal, Medical, and Executive	10,680	$27.76	57,745.00
Waiters and Waitresses	10,650	$19.43	40,429.00

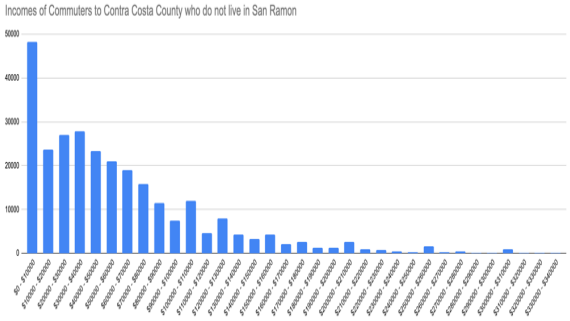

Figure 7. Incomes of Country Costa workers who do not live in San Ramon

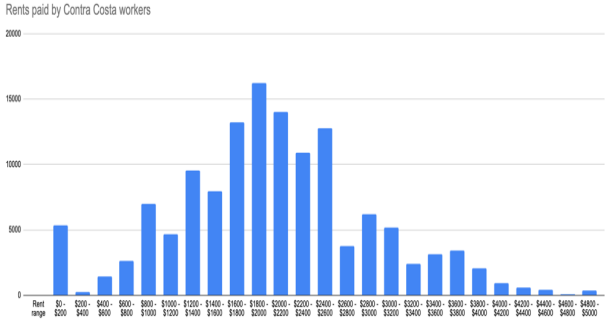

Figure 8. Rents paid by people who work in Contra Costa county

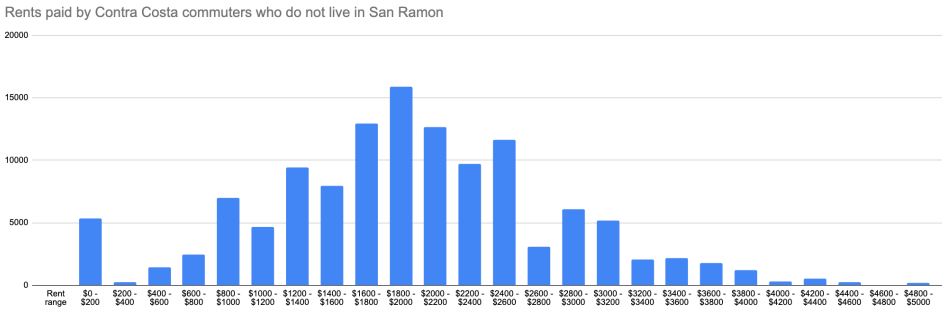

Figure 9. Rents paid by Contra Costa county commuters

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

Table 2. Median rent, median income, and approximate commute time for the seven largest feeder areas for the San Ramon labor market

City	Median income	Median 1 Bedroom Rent	Approximate commute time to San Ramon
Vallejo	$33,758	$1,550	35 Minutes
Benicia	$51,978	$1,550	30 Minutes
Livermore	$54,346	$2,072	20 Minutes
Pleasanton	$63,382	$2,515	15 Minutes
Dublin	$72,946	$2,922	11 Minutes
Fairfield	$37,636	$1,750	44 Minutes
Suisun	$34,559	$1,675	45 Minutes

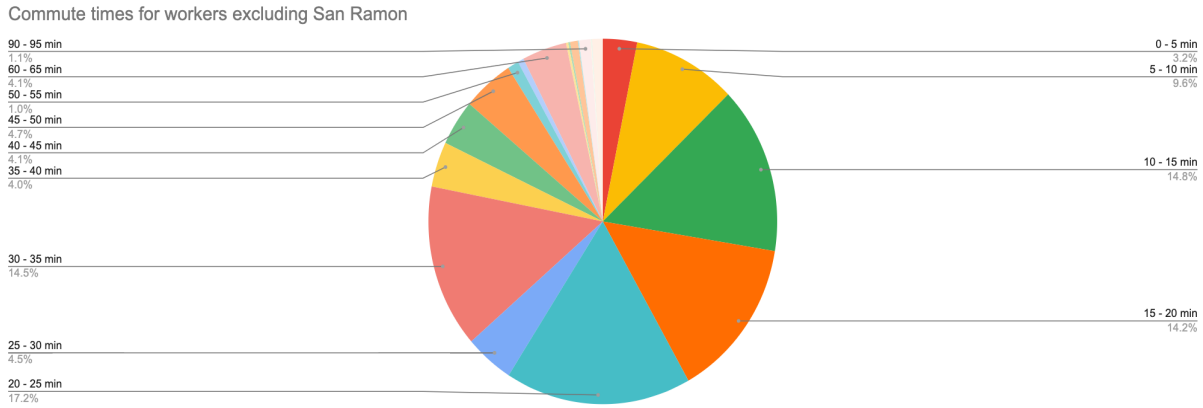

Figure 10. Distribution of commute times for people who commute to Contra Costa (CC) county

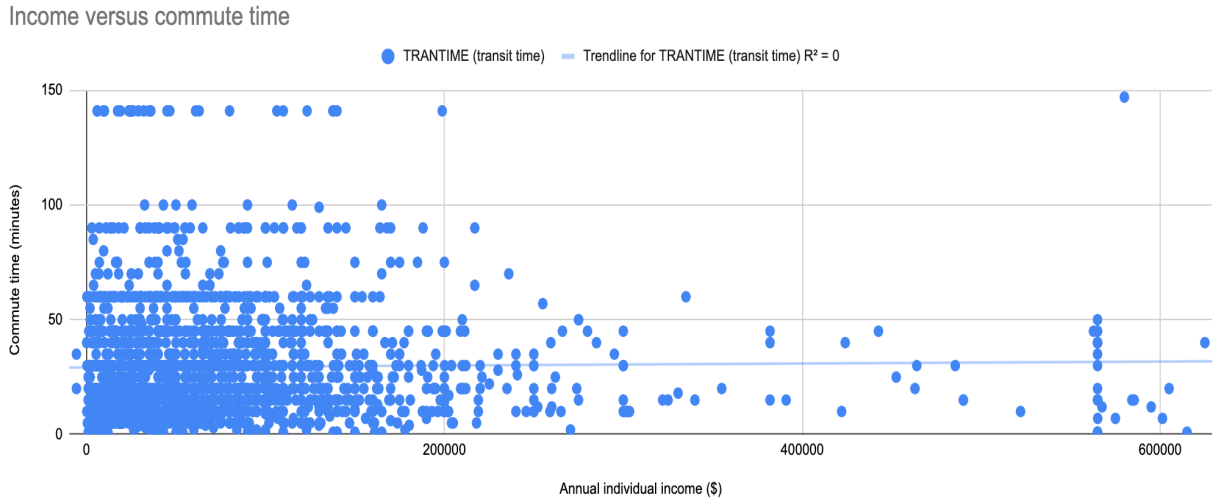

Figure 11. Income and Commute time among Contra Costa commuters

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

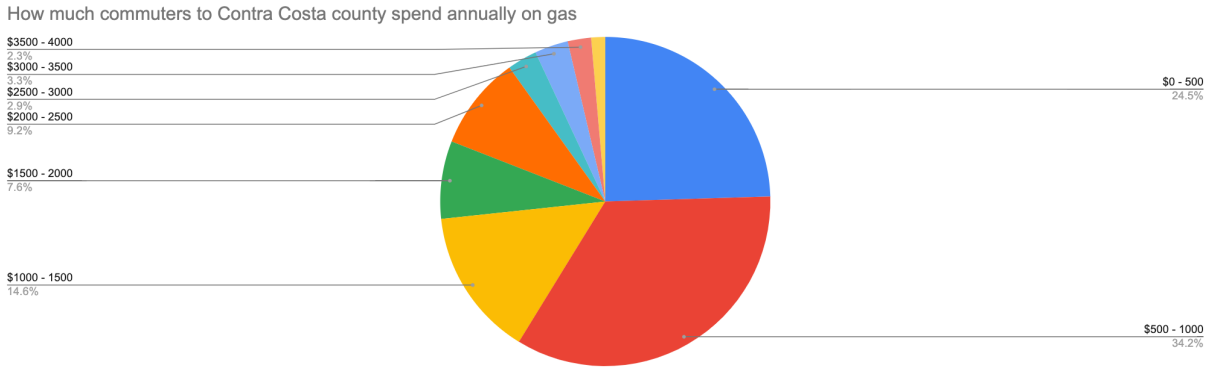

Fig 12. Annual amount spent on gas by commuters to Contra Costa County

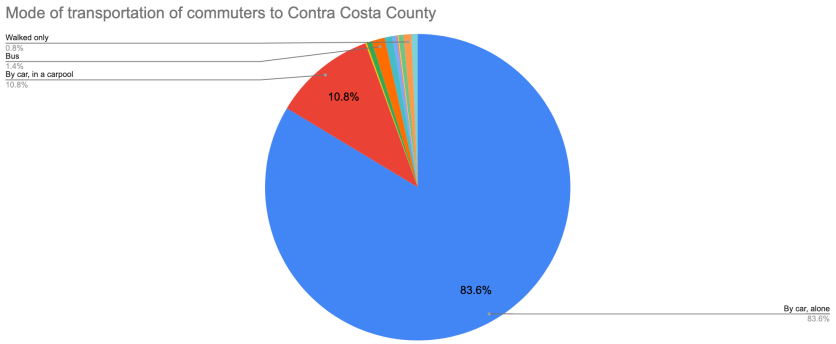

Fig 13. How commuters to Contra Costa County get to work

Table 3. Income limit, income minimum to avoid rent-burdened status, and percent of CC overall labor force and commuting workforce in each category

Housing type	Income limit (1 person)	Minimum income to avoid rent burden	Minimum income to pay no more than 44%* of income in rent	Percent of Contra Costa labor force eligible and not rent-burdened	Percent of Contra Costa commuters eligible and not rent-burdened	% of Contra Costa labor force eligible and spending no more than 44%* of income on rent	% of Contra Costa commuters eligible and spending no more than 44% of income on rent
50% AMI	$49,975	$49,960	$34,064	0%	0%	11.92%	14.66%
80% AMI	$79,960	$79,960	$54,518	0%	0%	16.37%	21.19%
120% AMI	$119,940	$119,960	$81,790	0%	0%	13.96%	15.35%

* Bay area average percent spent on rent

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

Table 4. San Ramon planning Meetings through July 26th, 2022

Meeting Year	Meeting Type	# of Meetings held	# of Times Project was mentioned	Total # of Public Participants across all meeting
2019	City Council	46	4	0
2020	City Council	42	7	19
2021	City Council	47	5	13
2022	City Council	26 (as of July 26)	0	0
2019	Planning Commission	25	4	3
2020	Planning Commission	27	8	48
2021	Planning Commission	28	9	14
2022	Planning Commission	20 (as of July 19)	3	5
2019	Architectural Review Board	13	0	0
2020	Architectural Review Board	12	0	0
2021	Architectural Review Board	15	0	0
2022	Architectural Review Board	8 (as of July 14)	0	0
2019	Transportation Demand Management	6	0	0
2020	Transportation Demand Management	6	0	0
2021	Transportation Demand Management	7	2	1
2022	Transportation Demand Management	4 (as of July 18)	0	0
2019	Housing Advisory Committee	4	1	0
2020	Housing Advisory Committee	5	1	0
2021	Housing Advisory Committee	6	0	0
2022	Housing Advisory Committee	4 (as of 5/22)	0	0
TOTAL		351	44	103
Per meeting averages			.125 mentions per meeting	.293 participants per meeting

Note: Table was made by tabulating publicly posted meeting notes for the City of San Ramon's City Council, Planning Commission, Architectural Review Board, Transportation Demand Management team, and the Housing Advisory Committee.

4. Discussion

4.1 Existing Housing Stock in San Ramon

San Ramon's housing stock is generally high in price. The median home costs $1,600,500; over half of the city's homes are valued at over $1 million, and another third are valued at over $750,000. Fewer than 2,500 homes are valued at less than $750,000, with only about 500 homes worth less than $300,000 (Fig 1). Despite their high price tags, home values in the area continue to rise quickly, further reducing the likelihood of a market-based solution to the city's lack of affordable housing (U.S. Census Bureau, 2020). Between 2021 and 2022, home prices went up 20.4%, and home appreciation the last 10 years hit 128.5% (Redfin, 2022). While home ownership in the area thus clearly requires significant financial resources, rental units are scarce: about 70% of units are owner-occupied. San Ramon does host several affordable housing facilities, but many of them cater to vulnerable populations like seniors

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

and people with disabilities (City of San Ramon-Housing Element, 2022). These populations are unlikely to provide a large proportion of the central city low-income workforce that would be best served by a 'walkable' center-city housing development.

4.2 San Ramon's Labor Market and its Resident Versus Commuter Workforce

San Ramon's residents are disproportionately white-collar workers at the apex of the economy; nearly 7 out of 10 of San Ramon working residents are employed in high-paying professional or managerial occupations, as shown in Figure 3 (U.S. Census Bureau, 2020). Many of these workers do not work in San Ramon proper: 62% of employed people who live in San Ramon commute to surrounding areas (travel more than 20 minutes to work) or work at home. (U.S. Census Bureau, 2020). San Ramon hosts a typical complement of businesses (retail stores, restaurants, personal service providers, etc.) to serve its affluent population, but only 8% of San Ramon's working population is employed in the service sector compared to nearly 80% nationwide (Ruggles et al., 2022). Accordingly, the service sector jobs located in San Ramon must be largely staffed by non-residents.

San Ramon's polarization reflects and produces a parallel disjuncture in the incomes of people who live in San Ramon as compared to the incomes of people who *work* in San Ramon. The majority of San Ramon's households earn more than $200,000 per year, nearly three times as much as the median household income for the United States as a whole (Fig 5). Only about 10.1% of residents earn a salary consistent with a low-wage service sector or central business district job in the area as described in Table 1.

State data describing the labor market for Contra Costa County -- the smallest geographical unit available, and the county in which San Ramon is located -- shows that the salaries of the area's *workforce* are distributed quite differently from those earned by San Ramon's residents. Most workers earn less than $65,000, and the majority earn less than $40,000 (Fig 5). Figure 6 shows a comparison between San Ramon's household incomes and the incomes of people who work in the area; while due to Census data limitations, the comparison is not quite perfect -- San Ramon residents who work within their county, for example, are reflected in both distributions, and county-level data is individualized whereas San Ramon data is at the household level -- the contrasting shapes of the two different distributions remain noteworthy.

This income polarization between workers and residents complicates the challenge of building affordable housing in high-income areas according to current state regulations and also speaks to broader challenges of income inequality across the country and the post-industrial and developing world. Employment for people who work in San Ramon itself naturally reflects a high proportion of service sector, customer service, and lower-level office work, the characteristic central business district labor market of a post-industrial city (Pierson, 1998; Nelson & Lorence, 1985). Indeed, the CityWalk development plans to expand rather than diversify this ecosystem; the commercial areas it proposes consist of new retail space and a new hotel.

Market-rate salaries for these new employees, however, will remain significantly below the wages of San Ramon residents and thus too low for even a 50% or 80% AMI apartment. The median cashier earns only about $38,000 per year, and the median retail worker earns only slightly more -- about $43,000 a year. The janitors and cleaners the new hotel might employ typically earn about $45,000 a year (Table 1). These salaries exceed state and national averages for similar jobs in part due to the high-income nature of San Ramon. Nevertheless, the extent of wage polarization in the area raises a question as to what types of housing units might actually be within the reach of these workers, most of whom do not live in San Ramon.

Indeed, about a quarter of the people who work in Contra Costa County commute from outside the county, coming primarily from Solano and Alameda counties, though many commute farther (Ruggles et al., 2022). As suggested by the divergent distributions of San Ramon's household incomes and the jobs offered by its labor market, commuters to Contra Costa County (the smallest available geographical unit for this dimension on the American Community Survey) have much lower incomes than San Ramon residents (Fig 7). More than half earn less than $50,000 per year, and a quarter earn less than $20,000.

American Community Survey data also show that these commuters currently pay rents significantly lower than those in San Ramon, although perhaps still more than they can comfortably afford (Fig 9). Removing those commuters

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

who live within Contra Costa County limits skews the distribution further, clearly concentrating rents on the lower end of the scale.

4.3 Communities that provide housing stock for low-income commuters and the consequences for commutes

Almost 90,000 workers commute into Contra Costa County, primarily from Alameda, Solano, and San Joaquin counties, though significant numbers commute farther (Employment Development Department, State of California, 2020). The median salary for low-wage workers in San Ramon is comparatively higher, in many cases, than that offered for similar positions in the rest of the country, but the high prices in San Ramon mean many workers can only afford rents in surrounding towns. A look at the seven largest feeder areas to the San Ramon labor market outside of Contra Costa County reveals that they have noticeably lower median incomes and rents than San Ramon but simultaneously impose significant commuting burdens for their San Ramon employed residents (Table 2).

This pattern fits with a long-established finding in the literature that socially and economically disadvantaged workers often bear a higher commuting burden (Roberto, 2008; Dodson et al., 2020). It also complicates the hypothesis based in economic theory that longer commutes should correspond to higher incomes since workers accept lower incomes in exchange for a shorter commute or demand a higher income exchange for a more onerous one. While this relationship has been found in research like Carra et al. (2018) and Zhu et al. (2017), it presumes the availability of housing at a wide range of prices and jobs at a wide range of wages throughout a geographical area.

Analysis of American Community Survey data from 2020 shows that generally, commute times for workers who work in Contra Costa County are not dramatically higher than commute times elsewhere in California or in the rest of the United States. Most people commute fewer than 25 minutes. However, a significant proportion of the workforce -- nearly a quarter -- commutes at least 40 minutes, and about one in ten commutes at least 50 minutes to work (Fig 10). Long commutes, especially in heavy traffic conditions like those that often persist in the area, incur environmental costs like air pollution, noise pollution, and carbon emissions as well as financial and personal burdens for commuters; the CityWalk development understandably seeks to create a pedestrian-friendly mixed-use zone in the context of this broader landscape.

Figure 11 charts commute times as they correspond to the incomes of respondents to the 2020 American Community Survey who work in Contra Costa County. No clear relationship of any kind emerges from the data, which has a correlation coefficient of 0. However, the commuters with the longest commutes are disproportionately among the lower-income respondents, and with a single exception, all of the very highest income commuters have commutes less than one hour in duration. At least in this labor market, lower-income workers are not being partially compensated by a shorter, less burdensome, or less expensive commute.

Survey data also shows that commuters spend a significant amount of money on gas, further lowering their effective salaries (Fig 12). Nearly half spend more than $1,000 a year on gas, and more than a quarter spend more than $1,500 per year, nearly 5% of wages for the median service-sector workers described in Table 1.

Nevertheless, public transportation and other non-automotive means of travel appear to be out of reach or impractical for most commuters. A breakdown of commuters' modes of transportation show that only about 5% travel to work without a car, and that the vast majority of drivers drive alone (Fig 13). While several transit operators serve the area -- the County Connection Bus (CCCTA), Tri Delta Transit, WestCat, AC transit, and Bay Area Rapid Transit (BART) -- the area's public transportation infrastructure is poorly integrated (Contra Costa Health Services, 2022). It also reflects a history of high-income communities leveraging concerns over growth and environmental impacts to keep out public transit and those who might use it to commute (Schafran, 2018).

4.4 Comparing CityWalk's New Housing Units to the Needs of Its Commuter Workforce

The CityWalk development plans on constructing 4,500 new units of housing sited in the center of San Ramon City over the 25-year life of the project. As per California state regulations, fifteen percent of the 4,500 (675) units built will be designated "affordable" for "low" and "very low income" households (City of San Ramon, 2020a). Nevertheless, the asymmetrical skews of San Ramon's resident incomes and labor force salaries complicate the nature

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

of this affordability. Given San Ramon's very high area median income, only individuals earning $49,975/year (who would pay $1249/month in rent) and $79,960/year (who would pay $1999/month in rent, respectively) would be eligible for and able to afford this housing (City of San Ramon- Housing Element, 2022). As shown in Table 3, the maximum income limits and rents for each type of apartment are set such that only people who are rent-burdened will be eligible to rent them; for the 120% AMI apartment, the income limit is set slightly *below* the salary needed to pay no more than 30% of yearly income in rent. If the income gap is expanded to include people who would be no more rent-burdened than the average Californian, who pays 44% of her income in rent (already a large proportion that significantly exceeds the national average of 27%), we find that the slice of the labor market eligible is still quite small. No more than one out of five workers is likely to find the "affordable" housing units "affordable" using even this elastic definition. Notably, CityWalk includes plans for 166,000 square feet of new retail and a hotel, both of which will require staffing by a largely low-wage workforce, only increasing the size and need of this population. However, the average retail sales associate in San Ramon earns about $20/hour, or about $40,000/year, nearly 20% below the "very low" income threshold and only about *half* of the "low" income level (Indeed, 2022a). Thus, a significant mismatch appears to exist between the workers who need affordable housing and the new housing planned. This research shows that the CityWalk development has indeed failed to meet the needs of its target population. Moreover, since it is built on a fundamentally characteristic dynamic of wealthy towns with service-sector economies, this pattern is likely to repeat itself in most similar municipalities.

4.5 San Ramon's Planning Process

The CityWalk development followed a relatively swift and-- in part due to the COVID-19 pandemic-- atypical approval process. First proposed in September 2019, it was approved just a year later, in September 2020, an unusually short period of time for a project of this scale. San Ramon is required by law to seek public comment and conduct community outreach meetings, and in fact, a public commenter (and former member of City Council) who submitted a lengthy public comment on the development as part of its environmental impact report (First Carbon Solutions, 2022) strenuously objected to the quick timeline. The relative scarcity of other inputs in that same document, however, suggests a related aspect of the problem: very few members of the public participated in the planning process, regardless of its duration, though meeting notes show that residents who did participate generally agreed that the project was rushed and was not given due-process, especially given its instigation during the global pandemic. While the fact that planning meetings were conducted virtually rather than in person might seem to have expanded the opportunity for public involvement, investigation of the minutes of these meetings showed that during those that were held as planned (a large number were canceled), public participation was extremely limited, and the CityWalk development was rarely mentioned (Table 4).

In the few Housing Advisory Committee meetings, minutes show, city officials conducted broad conversations about affordable housing, but CityWalk specifically was mentioned only once. Only the 2020 Planning commission meetings accumulated a significant number of public participants, though the per meeting average was still less than two per meeting (Table 4). In all of the meeting notes of the Architectural Review Board between 2019 and 2022, there was not one mention of CityWalk and the plans that Sunset Development, its developer, had for the project. Though the Review Board adhered to the mandatory 45-day waiting period before final approval, there was no input from the community during this waiting period (First Carbon Solutions, 2022).

In an interview, Senior Planner Cindy Yee noted that Sunset Development has been working with the city of San Ramon on its long-term plans for a very long time. Despite community concerns that the project got approved very quickly, many of which have surfaced in the media, Yee stated that there were meetings and conversations about the project between the city and Sunset Development before its official introduction, so the city "knew of Sunset Development's plans." These comments, however, indicate collaboration with the project's developer rather than broad-based public influence (C. Yee, personal communication, July 22, 2022). Indeed, in the public comments that do appear on the record, residents cited concerns about both traffic and housing, yet none of those concerns were addressed or mitigated; the project still was approved without modifications.

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

The lack of input from San Ramon's residents, though, constitutes only part of the city's participation challenges. As described earlier, the target population for the development's affordable housing units, in particular, would naturally consist of commuters to San Ramon not currently living within the community. There is little evidence, however, that the city reached out to non-resident stakeholders. Though a survey was posted on the city's website in October 2021 and publicized through its social media, it received only 296 responses, a tiny percentage of the more than 80,000 residents or more than 90,000 commuters (City of San Ramon-Housing Element, 2022). The events at which city staff were present to reach out proactively (e.g., a charity run, a farmer's market, a cultural event, a business expo, and an art festival) do not seem tailored to engage San Ramon's commuting center-city workers.

San Ramon's city planning documents indicate that the city reached out by certified mail to "surrounding jurisdictions and other housing-related stakeholders via e-mail and first-class mail for feedback and engagement," but jurisdictions themselves appear to be inadequate proxies for the low-wage work force that presumably represents the targets of the affordable housing units (City of San Ramon-Housing Element, 2022). San Ramon also reported "extensive outreach to property owners, non-profit housing developers, market-rate housing developers, homeless advocates, [and] the building industry," but those elements that might similarly lack focus on the needs of San Ramon's commuter workforce (City of San Ramon-Housing Element, 2022). Thus, this research indicates that the city did not successfully involve either resident or non-resident stakeholders. Though it is beyond the scope of this paper to suggest a causal relationship between the lack of input from the target population and the project's inability to serve its needs, such a relationship certainly cannot be ruled out.

4.6 CityWalk in the Context of San Ramon's Broader Infrastructure and Housing Goals

In the 2015-2023 RHNA cycle (Regional Housing Needs Allocation), the California Department of Housing and Community Development (HCD) apportioned 1,417 affordable housing units to the city of San Ramon: 516 in the "very low income" category, 279 in the "low-income" category, 282 designated "moderate income", and 340 reserved for "above moderate-income" renters. The city build out did not, however, meet the required 1,417 units (Association for Bay Area Governments, 2013). According to 2015-2020 Bay Area Building Permit Activity Report, San Ramon issued permits for only 25 "very low" income units (less than 5% of its allocation), 87 "low income" permits (less than one-third of its allocation), and 146 moderate-income units permits (about half of the number required) (Association of Bay Area Governments (ABAG) / Metropolitan Transportation Commission (MTC), 2020). When asked about the lack of sufficiently discounted housing for low-wage workers, the city's assistant planner Salmana Shah stated that the inclusionary housing policy includes no requirement to provide 30% AMI housing and that San Ramon has no plans to require buildings to provide very low income 30% AMI units in the future (S. Shah, personal communication, July 15, 2022). With regards to the broader issue of meeting RHNA quotas, city planning said that the city can allocate the land for building, but that it is difficult to make sure contractors and management companies follow through with building plans. Despite rules that require construction to be completed and monetary fines for failure to do so, there is no way, according to Cindy Yee, San Ramon's Senior Planner, for the city to enforce the unit construction once builders are issued the building permit; as a result, RHNA quotas that go unmet generate no coercive consequences (C. Yee, personal communication, July 22, 2022).

The RHNA cycle running from 2023-2031 (Association of Bay Area Governments, 2022a) has allocated San Ramon an additional 5,111 units of affordable housing. Despite the development's ambitions and scope, CityWalk's 675 units built over the next 25 years would represent only a drop in the bucket and, as noted, include no 30% AMI units (Association of Bay Area Governments (ABAG) / Metropolitan Transportation Commission (MTC), 2020). Indeed, the evidence suggests that the city is not anxious to approach the amount of construction the state envisages: San Ramon's government (unsuccessfully) appealed the state's housing allocation plan on July 9, 2020, arguing that the Association for Bay Area Government's forecasted development plan didn't take into account changes in San Ramon's jobs-housing balance, the annexation of two residences that added 1,286 units in 2016, and the fact that the land allocated for new units does not respect the historical use of the land (Association of Bay Area Governors, 2022a; Baum, 2021). Since the city could not meet its initial quota of 1,417 units and indeed sued to avoid meeting it, it is unlikely to meet the new, larger number.

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

San Ramon has laid out its own ambitious goals for reshaping its transportation infrastructure, also an important factor in the jobs/housing balance, as the research into its commuter population illustrates. In the City of San Ramon general plan, the city notes the high number of single-occupant vehicles in the area and lays out plans to remedy the problem, stating that businesses with over 50 full-time employees must have commuter benefits in the form of shared transportation (City of San Ramon, 2022a). San Ramon has also created a Transportation Demand Management (TDM) committee to make recommendations to the staff and City Council in furtherance of the goals of alleviating traffic and curtailing greenhouse gas emissions. In the case of CityWalk, however, it is not clear the committee has sufficiently addressed either the new or existing challenges to the area's transportation infrastructure.

The CityWalk project will occupy 135 acres of San Ramon's land, and given its 4,500 residential units, 169-room hotel, and 170,000 square feet of retail (City of San Ramon, 2020a), the toll it will take on public roads and transportation will be profound. The city documents for both Bishop Ranch, the development next to CityWalk which shares owners, promises tenants will be able to access free or subsidized Connection C bus service with direct and local express connections to Walnut Creek BART, Dublin/Pleasanton BART, Pleasanton ACE Train, and regional Park and Ride lots (Bishop Ranch, 2018). These steps may help to somewhat reduce the number of single-occupant vehicle rides, but the bus and train times cover "peak hours" (6:30-9:30 AM and 3:30-7:30 PM) only (County Connection, 2022), so they will serve only people working a traditional 9 to 5 job, thus excluding a large number of shift workers (e.g. many security guards and retail employees) or restaurant workers. Additionally, the buses do not run on the weekends, when many low-wage workers work since customer-facing establishments are often open. According to the Environmental Impact Report, all tenants will be given a free bus pass (First Carbon Solutions, 2022). Further exacerbating the limitations of these programs, new incentives to use public transportation may be outweighed by the "several" new parking facilities also planned for CityWalk; research suggests that new parking spots actually encourage more automotive traffic (McCahill, 2016; Weinberger, 2008). Despite these complexities, public transportation needs and strains on infrastructure due to the CityWalk development, meeting minutes show, have not yet been discussed. The new development was mentioned only twice in three and a half years of TDM committee meetings (Fig 18).

5. Conclusion

The housing created by the CityWalk development and the ways in which this development serves as a case study for similarly situated localities make these questions relevant for a wide variety of stakeholders. The nature of the CityWalk development and the housing it offers certainly affects current residents of San Ramon and might inform how they envisage a walkable, mixed-use downtown, but it also concerns the potential new residents of these housing units, who, if the city's goal of creating a town center where people both work and live is to be taken seriously, would staff both the new and existing retail and hotel amenities. This research shows that due to the high AMI of San Ramon and the income polarization of its residents compared to its workforce, California's regulations around affordable housing do not actually serve area workers. The affordable units envisioned both exclude the overwhelming majority of the city's residents, whose incomes exceed the legal limits, and price out the city's central business district workers, all of whom would have to take on substantial if not prohibitive rent burdens to live in them. City planners in San Ramon might consider the results of this research as they flesh out the CityWalk development and reflect on the populations they seek to serve.

Further, though, the question of how developments like CityWalk address the needs of high-income areas that attract low-income workforces (as shown in the scholarly work of researchers like Mollenkopf and Castells (1991) and Pinch (1993)) bears on the economies and housing stocks of similar cities in California and around the world. Policymakers at the state level might want to re-examine both the proportional requirements (15% affordable) and income levels (120%, 80%, 50%, and 30% AMI) in the context of an economy that is moving farther away from a normal distribution of income with a sizable middle class and towards a bimodal distribution of "haves" and "have-nots," (Hoffman, 2020) which might result in very few potential renters who both need and can afford an 80% or 50% AMI "affordable" housing unit. Perhaps standards based on larger labor market areas or even the state AMI would be more effective. Alternatively, income levels could be based on the 30th percentile worker, who generally earns

Vol. 2024 (1) 1 – 17
ISSN 2688-3651 [online]

substantially less than 30% of the AMI of an area like San Ramon. City planners and state and local governments across the United States and the globe should think critically about how best to serve the people who need affordable housing.

Finally, the question of public participation -- classically analyzed in the seminal 1969 work "A Ladder of Citizen Participation" by Sherry Arnstein -- is relevant to both government officials and the public at large. This research shows that San Ramon did not successfully engage either the city's citizens or the population that might have benefitted from living nearby their places of work in a "walkable" central city. For democracies to function, planners need to account for the needs of a diverse set of communities with varying barriers to civic involvement. As Arnstein herself suggested and as the work of scholars like McElroy and Szeto (2017) and Anguelovski (2016) has pointed out, the most vulnerable communities often lack the time, information, and/or faith in their local government to participate in even good-faith offers of communication. Some scholars like Sennett (2018) have suggested that bottom-up, technology-driven mass-participation in a so-called "smart city" might help to solve this problem, though this approach brings its own challenges, and legislative solutions that require local governments to consider a broader range of stakeholders might work better. The question of how governments serve constituents' needs when those needs and the solutions are extremely complex -- and when the bounds of the constituency itself might be under debate -- is both a timeless and timely concern.

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Journal of Research High School

Vol. 2024 (1) 18 – 25
ISSN 2688-3651 [online]

Correlation Analysis: How Over-Consumed Ingredients Influence Carcinogenesis in Asian Populations

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Abstract

The human immune system consists of a complex network of cells, tissues, organs, and substances that help the body protect itself from infectious diseases. Simultaneously, the immune system cells can recognize and remove damaged or abnormal cells with the potential to become cancerous. Early Greek physicians discovered that maintaining a healthy immune system is crucial to survival and unhealthy food consumption can undernourish the immune system and cause a state of failure. In a weakened state, the body can be infiltrated by several harmful pathogens. Specifically, Asian populations in Mongolia, Bangladesh, the Maldives, and Japan have higher rates of liver, oral, nasopharyngeal, and stomach cancer cases, which all stem from the body's digestive system. These cancers can form when a balanced diet is disrupted. Commonly eaten foods in these countries have been proven to impair the immune system's ability to attack cancer cells. More research is needed to fully understand the relationship between per capita food consumption of commonly consumed foods and cancer rates, and understanding this relationship could allow these populations to avoid overconsumption of harmful ingredients. This research paper examines the correlation between the most consumed foods in these specific countries and the respective high prevalence of cancer. The findings are promising and can be used to spread awareness on this issue in hopes that preventative action is taken to have populations reduce their consumption of such foods.

Keywords: Digestive Cancer, Nutritional Science, Oncology, Immunology, Correlation Analysis, Asian Populations

1. Introduction

The relationship between food and health has always been complex. Ancient Greeks believed that health was dependent on diet. The health and strengthening properties of herbs made them staples of the ancient Greek regime (Bramen, 2009). Most people now aim for healthier eating habits because our bodies rely on food for development. Scientifically, a primary hypothesis linking nutrition and health has to do with disruption to the immune system. The immune system allows the body to fight transmissible diseases and, put simply, is a human's protective armor. Nutrients can help natural resistance in several ways: serving as antioxidants, boosting immune cell activity, and producing necessary antibodies that assist our defense mechanisms. On the other hand, nutrient deficiency can significantly alter the body's immune responses (Bramen, 2009). Figure 1 illustrates how with the continued consumption of unhealthy foods and overall poor nourishment, the immune system's capability becomes damaged, making it more susceptible to bacterial, viral, and other infections.

Thus, select foods may affect the likelihood of health problems like cancer through the immune system. Focusing on improving the immune system with nutrient-filled diets could have promising effects on public health. In fact, around 40% of cancer cases could be prevented by focusing on nutritional improvement in patients (WCRF International, 2021).

Vol. 2024 (1) 18 – 25
ISSN 2688-3651 [online]

Previous research has expanded on the Greeks' discoveries, as epidemiological studies as early as the 1960s showed that cancer rates varied widely between populations and connected the differences to diet (Key et al., 2020). This was supplemented by experiments in animals that showed that cancer rates could be altered by manipulating diet and how energy intake causes a general reduction in cancer development (Key et al., 2020).

This paper makes the following contributions: (1) Determines the correlation between per capita consumption of commonly consumed foods in a country and the country's rate of certain cancers, (2) Examines whether the commonly consumed food in countries based on per capita consumption with the rates of digestive system cancers can be compared to the other countries with similar rates, and (3) Proposes future potential steps to prevent the high rates of cancer in these countries from increasing. As supported by this paper, the national cuisine of certain Asian countries contributing to damaged immune systems could be a reason behind their increasing cancer rates. We hypothesize that there will be a moderate to high correlation for each of the respective cancers and that this correlation will be observed consistently with other highly-rated countries.

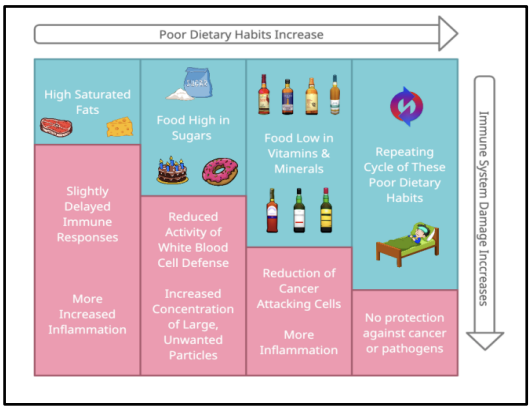

Figure 1. Dietary Habits versus Immune System Damage: The diagram shows how an increase in poor dietary habits causes magnified disruption to the immune system. This image is adapted from Chaari et al.(2020) Importance of Dietary Changes During the Coronavirus Pandemic: How to Upgrade Your Immune Response. Frontiers in Public Health.

If the findings of this study are promising, they will serve as a powerful catalyst for raising awareness about this critical issue. We hope to encourage communities and countries alike to reconsider their food choices and take proactive steps toward reducing their consumption of these detrimental foods. Ultimately, this concerted effort can lead to improved health outcomes in terms of cancer rates in society as a whole.

2. Materials and Methods

2.1. Description of Cancer Rates and Sources

The cancer rates in four Asian countries – Mongolia, Bangladesh, Maldives, and Japan – was extracted from data presented in the World Cancer Research Fund International Report (WCRF International, 2021). The data includes 18.1 million global cancer cases from 2020. The cases were categorized by the type of cancer (excluding non-melanoma skin cancer). They showed the top 10 countries with the highest specific age-standardized rate (ASR) – the rate of cancer that would be observed in a country with a standard-aged population. The ASR creates standardized cancer rates using the same age distribution which is crucial when comparing populations that differ in the countries' age distribution since age has a powerful influence on cancer rates. Based on the WCRF data, Asian countries typically had the highest ASR for liver, oral, nasopharyngeal, and stomach cancer. The countries marked in green below in Table 1 have one of the highest rates of these four cancers and reflect overall Asian populations. Thus, they were used for further analysis in determining the correlation between country level food consumption patterns and the cancer rate in that country.

Table 1. Highest Age-Standardized Rates of Digestive System Cancers in Different Countries: The numbers represent the age-standardized rates (ASR) of the respective cancers. The countries in green were chosen for the study based on their status as Asian countries and a high ASR.

Liver Cancer	Oral Cancer	Nasopharyngeal Cancer	Stomach Cancer
Mongolia: 85.6	Papua New Guinea: 25.7	Brunei: 9.9	Mongolia: 32.5
Egypt: 34.1	Bangladesh: 21.0	Maldives: 7.4	Japan: 31.6
Laos: 24.4	Romania: 17.2	Indonesia: 6.8	South Korea: 27.9
Cambodia: 24.3	Hungary: 17.2	Malaysia: 6.4	Tajikistan: 23.4

Vol. 2024 (1) 18 – 25
ISSN 2688-3651 [online]

2.2. Description of Per Capita Food Consumption by Country and Nutrition Data

Food consumption per capita for each of the four studied countries was obtained using national diet and nutrition databases from various publicly-available sources. These commonly consumed foods were taken into consideration to determine the link between a country's cuisine and rates of four cancers.

Using existing studies on the health impacts of over-consumed ingredients within each country's cuisine allowed for the formation of educated guesses concerning how certain ingredients could boost or impair an immune system's ability to combat cancer. In addition to analyzing the selected countries, the other three countries that showed similar cancer rates were studied to determine whether the highest-consumed foods at a per capita level were correlated with the respective cancer.

3. Results and Discussion

3.1. Liver Cancer

Mongolia showed the highest ASR for liver cancer in the world and in comparison to Asian countries. Liver cancer is characterized by the growth of cancer cells in the liver. The liver is an organ on the upper right side of the body that stores nutrients and filters out waste (Villines, 2020). The most commonly consumed foods in Mongolia include meat, vegetables, and noodles. Furthermore, the country is regarded to have, by far, the highest consumption of sheep and goat meat in the world, with the average Mongolian eating 50kg annually (Helgi Library, 2016).

Unfortunately, red meat is associated with an increased risk of liver cancer. A number of mechanisms could explain these findings. First, it is hypothesized that saturated fats, a macronutrient found in red meat, can delay immune responses to cancer cells (Freedman, 2010). Secondly, due to the presence of aflatoxins, a potential carcinogen that has led to an increased risk of liver cancer. These aflatoxins are known to contaminate animal products such as meat through the animal consuming contaminated feed (National Cancer Institute, 2022). Another possible explanation is the polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) that are produced when red meat is heated at high temperatures. Both compounds are known to be cancer-inducing (Medical College of Wisconsin, 2018). Therefore, the overconsumption of red meat (specifically sheep and goat meat) is likely linked to Mongolia's high rates of liver cancer among its population.

To determine whether red meat truly is liver cancer-causing, the levels of red meat consumption in Egypt, Laos, and Cambodia were also studied due to the similarities in liver cancer ASR when compared to Mongolia. Since ancient times, Egyptians have been known to eat high amounts of red meat, specifically preferring beef, mutton, and goat meat (Ramadan, 2022). In tandem with Mongolia, it is likely that the high red meat consumption in Egypt is a driving factor in the country's high liver cancer rates.

In Laos, bushmeat makes up over 75% of the meals of households whereas domestic meat such as pork, beef, and sheep meat is eaten in lower quantities (Napasirth et al., 2020). Bushmeat is raw or minimally processed meat that poses a communicable disease risk (Center for Disease Control and Prevention, 2022). In the case of Laos, it is likely that bushmeat is what causes the high rates of liver cancer as a study determined viruses and the liver fluke Opisthorchis viverrini, commonly occurring in bush meat, to be the main cause of the high liver cancer incidence in Laos (Sitbounlang et. al, 2021).

Finally, in Cambodia, red meat consumption is known to be extremely high, where each person on average consumes 17.6kg of meat per year (Vireak, 2020). Comparing this to Mongolia's average annual meat consumption per person of 8.5kg, it is clear Cambodia consumes more red meat on average (United Nations, 2021). However, Cambodia still consumes less sheep and goat meat than Mongolia, so it is likely that sheep and goat meat are more cancer-causing than general red meat.

Overall, it is evident that in three out of the four countries, the high consumption of sheep and goat meat, as well as red meat in general, is associated with the high liver cancer rates.

Vol. 2024 (1) 18 – 25
ISSN 2688-3651 [online]

3.2. Oral Cancer

Bangladesh showed the second-highest ASR for oral cancer in the world and the highest amongst Asian nations, reaching an ASR of 21.0. The rates of oral cancer are rising, with more than 7000 people diagnosed each year (Sultana, 2014). Bangladesh has two risk factors related to high consumption — tobacco and rice.

The most common form of tobacco chewing in Bangladesh is known as betel quid chewing, consisting of Areca nut, betel leaf, catechu, zarda, and slaked lime (Malik, 2014). This tobacco contains harmful chemicals such as nitrosamines and benzene, which lead to cancer-causing mutations and abnormal cell growth when they come into contact with the tissues of the mouth and throat. This is likely why those who chew betel quid are at 4 times higher risk of developing oral cancer compared to non-smokers (Ahmed, 1990).

In terms of rice, it is the staple food of Bangladesh and Bangladesh has the highest per capita consumption of rice in Asia (Ali, 2020). Studies have revealed that rice is a major source of arsenic, which is known to be a substance that promotes the formation of cancer under the condition of long-term consumption (Columbia University, 2023).

To determine whether tobacco and rice truly contribute to high oral cancer rates, the levels of tobacco and rice consumption in Papua New Guinea, Romania, and Hungary were studied due to the similarities in oral cancer ASR when compared to Bangladesh. Papua New Guinea is currently among the top ten countries in the world in terms of tobacco consumption, with around 40% of the population consuming tobacco (Hou et al., 2015). This aligns with Bangladesh's high tobacco consumption rates. Furthermore, Papua New Guinea also has extremely high rice consumption rates among its population. This is likely why there have been rice price increases in Papua New Guinea since 99% of rice is imported to the country (Schmidt, 2021). Again, Papua New Guinea shows a similar per capita food consumption level for rice. It is likely that both tobacco and rice contribute to the high ASR of oral cancer in Papua New Guinea as it does in Bangladesh.

Romania has some of the highest rates of smoking – it is currently third in men and first in women amongst European countries and one of the top countries for tobacco use overall in the world (Euro News, 2023). Although in recent years its rates of tobacco use have been falling, it still has one of the highest tobacco consumption rates in the European Union. In terms of rice consumption, Romania consumes significantly more rice than other European countries (Landgeist, 2022). Although Romania's levels of tobacco and rice are much lower than in Asian countries, it is still among the top consumers of these goods in Europe, which is likely why it has the highest ASR for oral cancer among European countries.

Finally, Hungary is known to have about a third of its population smoking daily, which would explain why it has the fourth highest rate of oral cancer in terms of ASR (National Institute of Health, 2013). For rice consumption, much like Romania, Hungary also consumes far more rice than other Eastern European countries, and consumption rates have been continuously growing (Landgeist, 2022).

In conclusion, all four of the countries show a strong positive correlation between tobacco and rice consumption rates and their respective high ASR for oral cancer.

3.3. Nasopharyngeal Cancer

Globally, the Maldives showed the second highest ASR for nasopharyngeal cancer – abnormal cancer growth that stems in the back of the throat. The nasopharynx is an essential component of the digestive system by connecting the throat to other digestive organs (Villines, 2020). The Maldives' daily diet primarily consists of fish and plant foods. Fish meat is of particular importance, as Maldivians have the highest per capita consumption of fish in the world (RISE Seafood). This poses a major issue, as recently it was discovered that there are several contaminants in tuna, such as fungi and mercury, that can increase the risk of cancer (Swiers, 2022). The Maldives presents yet another case in which the likelihood of certain food consumption may lead to higher cancer rates.

Looking at Brunei, it is known that seafood is one of the principal sources of protein. Indeed, the per capita consumption of seafood in Brunei is one of the highest in the world as per data in 2021 (Department Of Fisheries, 2021). This could explain why Brunei has the highest ASR of nasopharyngeal cancer in the world.

Vol. 2024 (1) 18 – 25
ISSN 2688-3651 [online]

The case in Indonesia, with the third highest ASR of nasopharyngeal cancer, is slightly different from Brunei and the Maldives. Despite an increase in fish consumption within the country, fish consumption is still lower in Indonesia than in some other Asian countries (Anyanwu et al., 2023). There are likely other factors than nutrition contributing to the high nasopharyngeal cancer rates in Indonesia.

Fortunately, fish consumption in Malaysia confirms that nutrition may be a key reason behind its almost equally high ASR. Malaysians, in particular, consume fish at least once a day (Ahmad et al., 2016). In comparison to all Asian nations, the annual per capita fish consumption of Malaysians was the second highest after Japan and is ranked number five globally.

In summary, three of the four countries with the highest ASRs for nasopharyngeal cancer appear to have high fish consumption, which has been previously linked to increasing the risk of cancer.

3.4. Stomach Cancer

Stomach cancer rates in Japan are the second highest in the world, with an ASR of approximately 31.6. There are a few risk factors within Japanese diets that could possibly lead to these high rates.

First is salted foods. Due to high rates of Helicobacter pylori infection and increased intake of salted and smoked foods, several Asian nations, including Japan, have disproportionately high incidences of stomach cancer (Yang, 2021). However, Japan specifically is higher at risk as the Japanese generally consume more salt than people in other countries with lower per capita salt intake (Tanaka et al., 2023).

The second major risk factor is tea consumption. According to a study, the risk of stomach cancer according to the frequent intakes of total tea, green tea, black tea, and oolong tea was tested and showed a connection to high rates of stomach cancer (Sheerah et al. 2020). This confirms that the rates of stomach cancer can partially be caused by the high rates of salted/smoked foods as well as tea because stomach cancer is a multifactorial illness with a complicated interplay of genetics, lifestyle, and environmental variables (Lin, 2011).

In Mongolia, with the highest ASR of stomach cancer in the world, salt consumption rates are extremely high. In fact, the World Health Organization recommends that people consume no more than 5-6 grams of salt per day (World Health Organization, 2023). However, on average, Mongolians consume two-fold more salt than or around 11.5 grams of salt. Fortunately, current efforts are being made to limit salt consumption amongst the population and prevent increasing risks of a variety of diseases (Montsame, 2018). Furthermore, tea is known to be the most consumed drink in Mongolia and is seen as a staple drink, a medicinal potion, or a food supplement (Bamana, 2015).

For South Korea, with the third highest ASR of stomach cancer in the world, the amount of salt consumed exceeds the daily sodium intake recommended by the WHO as similarly seen in Mongolia (Pyo et al., 2023). Tea consumption is the opposite, however, with Korea having a relatively low tea consumption despite tea drinking being a part of Koreans' daily lives (Hyun-kyung, 2018).

Finally, Tajikistan has unhealthily high salt consumption rates. In fact, the World Health Organization in 2017 found that the dishes surveyed in the country contained up to 124.5% of the maximum recommended daily intake of salt. However, Tajikistan does not have very high tea consumption rates, with its per capita consumption being 54th in the world (World Population Review, 2023).

Based on these results, salt consumption has a much stronger correlation to high stomach cancer ASR as confirmed by all four countries. Tea consumption has a much weaker correlation, with only two of the three countries showing high tea consumption rates.

4. Conclusion

In conclusion, our comprehensive examination of digestive cancer rates in various countries has shed light on intriguing correlations between dietary habits and the prevalence of these cancer types. While the findings reinforce some well-established connections, they also reveal nuances that call for a more critical evaluation and targeted recommendations. For liver cancer, there appears to be a fairly strong correlation between sheep and goat meat and cancer rates as confirmed by three of the four countries with the highest liver cancer ASR. Second, there is a highly

Vol. 2024 (1) 18 – 25
ISSN 2688-3651 [online]

strong positive correlation between oral cancer ASR and tobacco and rice consumption in all four of the countries examined. Third, nasopharyngeal cancer rates in the Maldives, Brunei, and Malaysia highlight the large potential role of high fish consumption in cancer risk. Finally, salt consumption appears to play a strong role in the high stomach cancer rates in Japan, Mongolia, South Korea, and Tajikistan. In light of these findings, it is evident that a one-size-fits-all approach to cancer prevention may not be sufficient. In the cases where only three of the four countries confirmed our hypothesis, further research is needed to confirm whether food consumption truly contributes to the respective cancer risk. From there, it will be important to identify potential mitigating strategies in hopes that preventative measures can be taken within the countries with the highest cancer rates. Collaborative efforts involving healthcare professionals, policymakers, and the community are needed to develop targeted and tailored strategies for cancer prevention and awareness campaigns in each country. Future research should delve deeper into other possible genetic and environmental factors contributing to cancer risk in these regions. Understanding the interplay of genetics, lifestyle, and environmental variables will enable more precise and effective cancer prevention strategies.

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Vol. 2024 (1) 18 – 25
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Vol. 2024 (1) 18 – 25
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Journal of Research High School

Vol. 2024 (1) 26 – 30
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Increasing the Accuracy of Early Parkinson's Disease Diagnosis with Slow Saccadic Intrusions

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Abstract

Parkinson's Disease is a chronic neurodegenerative disorder that causes uncontrollable movements in the body, including tremors and impaired balance. As of today, there is no cure for Parkinson's Disease. While there are treatments such as Levodopa that can be used to slow the progression of the disease and control certain symptoms, it may not always be effective. Therefore, early detection of the disease is crucial. However, as the diagnosis of Parkinson's Disease heavily relies on subjective physician judgment and rarely on clinical tests, detecting Parkinson's Disease at an early stage is often challenging, inaccurate, and inconclusive. One possible way to detect Parkinson's Disease both early and accurately is through the detection of abnormal saccadic intrusions – in this case, slow saccades. This study used Dai and colleagues' algorithm based on the implicit piecewise polynomial approximation model, which includes the nonlinear denoising step and basic velocity-threshold step that helps detect slow saccades with high precision, to prove how it could be used to diagnose Parkinson's Disease early.

Keywords: Parkinson's disease, Neurodegenerative disorder, Saccadic intrusions, Implicit piecewise polynomial approximation

1. Introduction

Parkinson's Disease progresses as dopaminergic neurons located in the midbrain stop functioning properly (Alexander, 2004). As a result, this brain disorder can cause failures in the motor system, leading to bradykinesia, tremors, rigidity, postural instability, and more (Mazzoni et. al., 2012). Today, Parkinson's Disease is the second most prevalent neurodegenerative disease and does not have a cure available for use (Alexander, 2004). Therefore, the early diagnosis of Parkinson's Disease is crucial in slowing down and minimizing the disease progression. Moreover, detecting Parkinson's Disease early can increase the effectiveness of treatments and reduce symptoms like dyskinesia (Murman, 2012). However, Parkinson's Disease diagnosis is known to be very subjective and difficult as the symptoms can vary widely among individuals. In fact, a study has shown that the accuracy of the early detection of Parkinson's Disease is only 58%, with 3.6% to 19% of Parkinson's Disease subjects found to be scans without evidence of dopaminergic deficit (Beach and Adler, 2019). In other words, even patients that were clinically diagnosed with Parkinson's Disease have shown normal findings on dopaminergic imaging, indicating that they are not likely to have Parkinson's Disease. Inaccuracies like such present in the early detection of Parkinson's Disease reiterates the urgent need to develop more effective methods to increase the accuracy of diagnosis.

Saccadic intrusions are involuntary rapid eye movements that interrupt fixation on a target. Abnormalities of saccades are a common symptom in the early stages of many movement disorders, including Parkinson's Disease (Termsarasab et. al., 2015). In fact, a recent study reveals that Parkinson's Disease patients have slower saccades compared to healthy controls, which means that accurately detecting the number of slow saccades can be a viable

Vol. 2024 (1) 26 – 30
ISSN 2688-3651 [online]

method in detecting Parkinson's Disease early (Fooken et. al., 2022). Slow saccades are caused by abnormal bursting, a neural activity characterized by repetitive fast spiking. Abnormal busting of basal ganglia neurons is present among Parkinson's Disease patients, in which there is an evident increase in neuronal firing following the progression of the disease (Lobb, 2014). However, saccades are often overlooked in the clinical diagnosis of Parkinson's Disease and detecting them require technical expertise.

This study explores the function and accuracy of Dai and colleagues' algorithm based on the implicit piecewise polynomial model and investigates its potential to be used as a tool to diagnose Parkinson's Disease early. Although there were algorithms developed in the past with the ability to detect normal saccades, they have all faced limitations when detecting slow saccades. However, the proposed algorithm has demonstrated the ability to accurately track slow saccades, showing its strong potential in being used to detect symptoms of early-stage Parkinson's Disease. Therefore, this study's objective is to highlight how the proposed algorithm's denoising step and velocity-threshold step should be applied in the clinical setting to provide an affordable, accessible, and objective method to diagnose Parkinson's Disease.

2. Materials and Methods

Dai and colleagues have proposed an algorithm based on the implicit piecewise polynomial approximation model that contains two major steps: the nonlinear denoising step and basic velocity-threshold step. The denoising step is used to reduce the presence of noise and keep track of abrupt changes that occur with saccadic eye movement. This step that improves noise reduction is unique to this proposed algorithm and plays a significant role in increasing the accuracy in detecting slow saccades. Following that, the basic velocity-threshold step allowed the detection of saccades by applying the velocity-threshold step to the velocity of the estimated time series x. This step showed that the velocity is piecewise linear during saccadic intrusions and zero when fixed. The velocity must exceed 30 degrees/second to be considered a saccade (Dai et. al., 2021). While many accurate detection algorithms are already present, this algorithm is the only one that is designed to detect the movement of slow saccades, which makes it suitable as a method to increase the accuracy of early Parkinson's Disease diagnosis.

3. Results

Figure 1 reproduced below presented how the proposed denoising step successfully improved the reduction of noise in both the position and velocity of the saccades, ensuring a more reliable detection of saccades. Slow saccades are more easily influenced by noise compared to normal saccades with the same amplitude because of temporal differentiation in velocity-threshold step. While temporal differentiation is required to determine the velocity from the position data, it also amplifies noise, making reliable detection more difficult. (Dai et. al., 2021). Saccades are classified based on their velocity, in which it is considered a saccade when the velocity exceeds a threshold, and otherwise, it is a fixation. Moreover, lower velocity of slow saccades makes velocity thresholding less reliable due to the presence of noise hindering the detection process. Therefore, the denoising step in the proposed method is crucial.

The mean score of the proposed algorithm in detecting saccadic intrusions at different noise levels is 1.000 (Dai et. al., 2021). The perfect score revealed the extremely high accuracy rate of the algorithm when compared to previous algorithms for saccade detection. After the proposed denoising step, the root mean square error (RMSE) decreased, meaning there was a reduction in the average difference between the given model's predicted values and the actual values. This proves that the model's predicted values are highly accurate. Following the proposed algorithm, Zembly's algorithm demonstrated the second highest accuracy when detecting saccades under noise, outperforming other saccade detection algorithms. Yet, its mean score is 0.996, which is still lower than the proposed algorithm's perfect value of 1.00 (Dai et. al., 2021). Unlike the proposed method based on a velocity-threshold algorithm, Zemblys' is a machine-learning algorithm that uses various features extracted from the data, including Rayleigh test and i2mc, training a random forest model to classify saccades. Although machine-learning models are stronger in detecting saccades than hand-crafted algorithms like Nystrom's, it still struggles when it comes to slow saccades (Dai et. al., 2021). Moreover, while Zemblys' showed the ability to detect the normal saccades properly, it failed to precisely determine the end of

Vol. 2024 (1) 26 – 30
ISSN 2688-3651 [online]

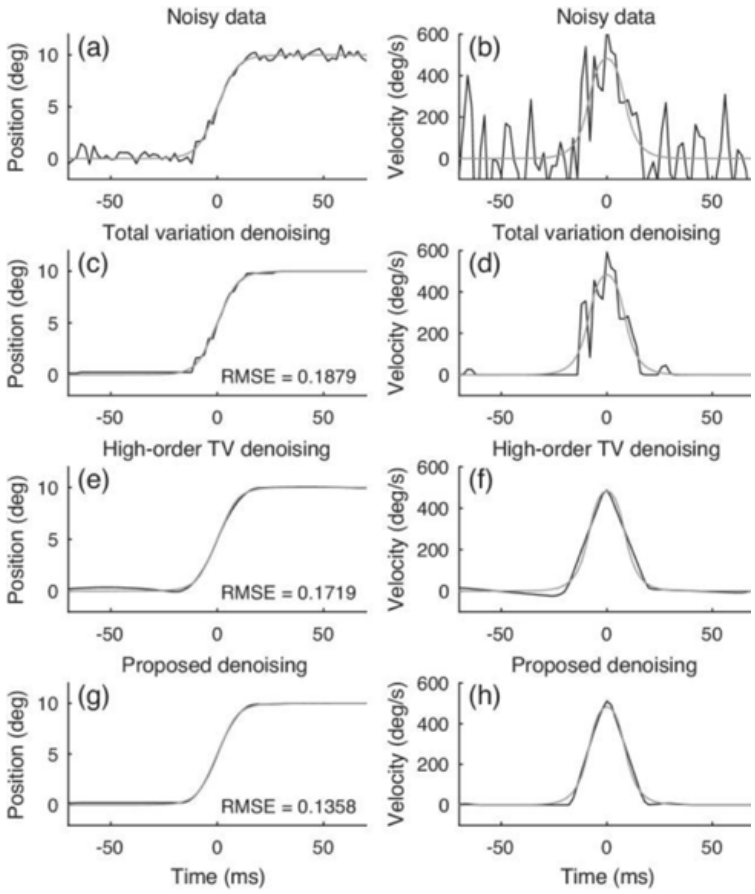

Figure 1: Noise signals after the proposed denoising step (Dai et. al., 2021).

Parkinson's Disease earlier with great precision.

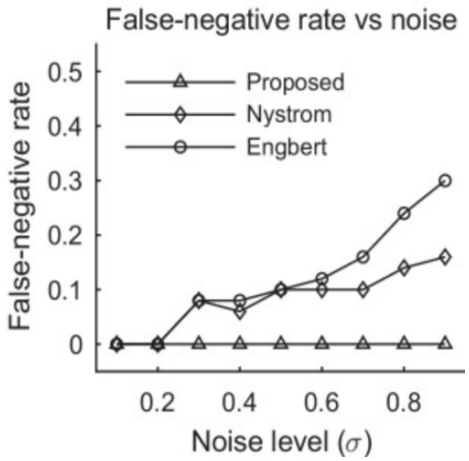

Figure 2: The rate of detecting false saccades among different algorithms (Dai et. al., 2021).

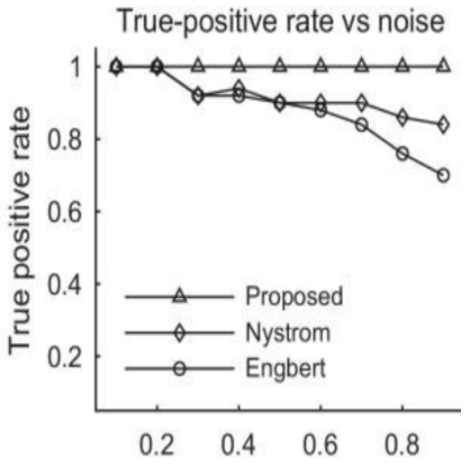

Figure 3: The rate of detecting true saccades among different algorithms (Dai et. al., 2021).

Not only does the proposed algorithm have a low false-negative rate, but it also shows a high true positive rate. As shown in figure 3, the proposed algorithm is the only one from the three that maintains a near perfect rate of 1

slow saccades, falsely classifying large slow saccades as small normal saccades (Dai et. al., 2021). With a significantly improved denoising system, the proposed algorithm based on the implicit piecewise polynomial approximation model can more accurately detect the movement of saccades and find abnormalities, thus, can be used as a potential tool for aiding the early diagnosis of Parkinson's Disease.

When compared to other detection algorithms, Dai and colleague's proposed algorithm showed an overall higher accuracy in detecting true saccades. Figure 2 revealed that the proposed algorithm maintains a very stable false-negative rate of 0, proving that the algorithm is fully capable of handling slow saccades, even under high noise levels. Other algorithms such as Nystrom and Engbert's algorithm detect false saccades more often than the proposed, especially with increasing noise levels. As the proposed algorithm showed a significantly higher accuracy rate when compared to pre-existing algorithms, it can be used to detect

Vol. 2024 (1) 26 – 30
ISSN 2688-3651 [online]

throughout increasing noise levels. This means, while the proposed algorithm near perfectly avoids inaccurately detecting false saccades, it can also detect real saccades, even under the presence of more noise. Altogether, with the algorithm's ability to detect slow saccades with high precision and distinguish between true and false saccades, it has a strong potential to increase the accuracy of early Parkinson's Disease diagnosis.

Below, Table 1 presented the summary of the proposed algorithm's high accuracy rate of detecting slow saccades compared to 10 pre-existing algorithms. Despite the increasing amount of noise level, the proposed algorithm outperforms the other algorithms with its perfect accuracy. In comparison, the other algorithms' accuracy rate tends to fall as the noise levels increase.

Table 1: Accuracy in detecting slow saccades under different noise levels using simulated time-series (500 samples/second) (Dai et. al., 2021).

	Proposed	**Zemblys**	**Nystrom**	**Konig**	**Engbert**	**VT**	**DT**	**HMM**	**Friedman**	**MST**	**KF**
$\sigma = 0.1$	1.00	1.00	1.00	0.96	1.00	0.18	0.78	0.23	0.98	0.63	0.49
$\sigma = 0.2$	1.00	1.00	1.00	0.96	1.00	0.4	0.51	0.18	0.85	0.5	0.04
$\sigma = 0.3$	1.00	1.00	0.96	0.96	0.96	0.47	0.27	0.28	0.45	0.49	0.04
$\sigma = 0.4$	1.00	0.98	0.97	0.96	0.96	0.58	0.27	0.04	0.21	0.22	0.04
$\sigma = 0.5$	1.00	1.00	0.95	0.96	0.95	0.61	0.26	0.04	0.07	0.15	0.04
$\sigma = 0.6$	1.00	1.00	0.95	0.95	0.93	0.59	0.27	0.04	0.02	0.11	0.04
$\sigma = 0.7$	1.00	1.00	0.95	0.94	0.88	0.62	0.26	0.76	0.03	0.07	0.04
$\sigma = 0.8$	1.00	1.00	0.92	0.93	0.84	0.55	0.27	0.76	0.00	0.06	0.04
$\sigma = 0.9$	1.00	0.98	0.91	0.95	0.78	0.54	0.25	0.76	0.00	0.04	0.04
mean	1.000	0.996	0.956	0.950	0.921	0.504	0.348	0.342	0.289	0.252	0.089

4. Discussion

This study introduced an algorithm based on the implicit piecewise polynomial approximation model and explored the relationship between slow saccades and Parkinson's Disease. Although Dai's algorithm showed a high performance overall by detecting saccades perfectly under different noise levels through the denoising step and maintaining a low false-negative rate, it still has drawbacks that must be addressed. Currently, the proposed algorithm is not intended to track data with nystagmus or smooth pursuit eye movements. However, as smooth pursuit eye movements are a common abnormality seen in Parkinson's Disease patients (Dai et. al., 2021), it is crucial to modify the system in the future to reprocess the data and consider the presence of various abnormal eye movements that may be present among the patients. Apart from Parkinson's Disease, the algorithm can potentially be used for the early diagnosis of other neurodegenerative disorders, including Alzheimer's Disease, progressive supranuclear palsy, or spinocerebellar ataxia type 2 (Jensen, 2019). This is because reduced saccade velocity is a frequent phenomenology that is seen in cerebellar diseases.

5. Conclusion

Today, nearly 1 million Americans are estimated to have Parkinson's Disease. As no cure is available for Parkinson's Disease, it is important to detect symptoms of Parkinson's Disease as soon as possible to receive proper treatment. To do so, saccadic intrusions must not be overlooked. Abnormal saccades are one of the few symptoms of Parkinson's Disease that appear in the early stages, thus, they can act as a visuomotor warning that the body is experiencing a problem. As the proposed algorithm's denoising step and velocity-threshold step shows success in remaining highly accurate in its detection of slow saccades even under the presence of noise, the study solidifies its strong potential to increase the accuracy of the early diagnosis of Parkinson's Disease. Currently, the cost of getting scans like DAT scan can cost anywhere from $2,500 to $5,000, making it expensive to order for the general public, especially elders from disadvantaged and isolated backgrounds. Thus, this research can not only help simply and speed up the diagnosis process for the clinicians, but also make it accessible and affordable for all patients.

Vol. 2024 (1) 26 – 30
ISSN 2688-3651 [online]

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Journal of Research High School

Vol. 2024 (1) 31 – 37
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Development of Environmental Justice Index for Water Quality in NY State Using Trihalomethanes

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Abstract

Trihalomethanes (THMs), which are likely to cause cancer in humans, are frequently detected as disinfection by-products (DBPs) in public water systems (PWS). A recent study revealed that regions with higher incomes typically had lower THM levels in New York State, potentially suggesting a socioeconomic disparity concerning the quality of drinking water. Expanding on this crucial discovery, our research sought to lead the development of an Environmental Justice (EJ) Index for evaluating drinking water quality, using THM concentrations as the primary metric. Our hypothesis centers on utilizing THM concentration as an environmental indicator to address issues of environmental justice, given the health risks associated with THMs and their susceptibility to socio-economic and environmental factors in communities. The step-by-step creation and practical application of an EJ Index for THMs has been demonstrated, highlighting the suitability of THMs as an EJ indicator. Through this comprehensive approach, our research aimed to initiate the inclusion of a water-quality related EJ index in the current U.S. EPA's thirteen EJ indexes which lack adequate representation of water quality in communities.

Keywords: EJ Index, Trihalomethanes, Disinfection By-Products, Socioeconomic Disparity, New York

1. Introduction

A national assessment spanning over three decades, from 1982 to 2015, examined drinking water quality violations in public water systems (PWS) and discovered that approximately 8.0% of PWS experienced health-based violations during this period (Allaire, 2018). Among these violations, a significant proportion, approximately 25%, were attributed to disinfection by-products (DBPs) (Allaire, 2018). Trihalomethanes (THMs), which belong to the class of DBPs, are known to form as a result of the chemical reactions between natural organic matter and disinfectants, such as chlorine, employed in the treatment of public drinking water (DeMarini, 2020). The U.S. Environmental Protection Agency (EPA) regulates four trihalomethanes including chloroform, bromodichloromethane, dibromochloromethane, and bromoform which are referred to as total trihalomethanes (or THM4) of which federal regulatory level is set at 80 μg/L (U.S. EPA, 2018). One of the key reasons THMs warrant attention is their classification as probable human carcinogens, supported by findings from experimental studies involving laboratory animals (Attias, 1995). Moreover, numerous epidemiological investigations have established associations between exposure to chlorinated drinking water, which often contains THMs, and the incidence of conditions such as rectal, colon, and bladder cancers (Costet, 2011; Hildesheim, 1998). This robust body of evidence underscores the significance of THMs as an environmental indicator for drinking water quality, as their presence not only reflects the prevalence of DBPs but also raises pertinent public health considerations, particularly concerning potential carcinogenic risks associated with long-term exposure.

Vol. 2024 (1) 31 – 37
ISSN 2688-3651 [online]

Elevated THM levels exceeding federal mandates were observed in the watersheds of three states (Arkansas, Nevada, and Rhode Island) characterized by lower median household incomes compared to the national average, while states with higher median household incomes (Delaware, New Hampshire, and Wisconsin) exhibited notably low THM levels (Karim, 2020). In a THM study conducted in middle Tennessee, it was noted that specific areas within each watershed, marked by lower median household incomes, demonstrated heightened levels of THMs in their drinking water (Guha, 2019). While this previous two studies did not demonstrate a significant correlation between THM levels and socioeconomic factors through data points from either U.S. states or major watersheds in Tennessee, the recent study by Lee and Park (2023) made a noteworthy discovery. It revealed a negative correlation between median household income and THM concentrations at the city (or village) level in the state of New York. This inverse relationship raises an important concern, suggesting the existence of socioeconomic disparities in terms of drinking water quality. As a natural progression from this pivotal finding, our research endeavored to pioneer the development of an Environmental Justice (EJ) Index tailored to assess drinking water quality using THM concentrations as a primary metric.

The primary objective of this article is to demonstrate the suitability of THMs as an EJ indicator for drinking water quality and to develop an EJ index, aiming to shed light on regions within New York State that might bear higher environmental burdens, potentially affecting vulnerable populations. Our methodology aligns with the EPA's established EJ Index development tool in EJScreen, which amalgamates both environmental and socioeconomic indicators to provide a comprehensive perspective on environmental justice (US EPA, 2023).

We hypothesized that the concentration of THMs can be used as an environmental indicator to discuss environmental justice because THMs can pose health risks, and their presence in drinking water can be influenced by a community's socio-economic and environmental conditions. We presented the detailed procedure for developing an EJ Index for THMs and its application. We compared the results of the previous correlation study between THMs and socioeconomic factors with the outcomes of the correlation study applied using the developed EJ index.

2. Materials and Methods

The average concentrations of total trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) in 2020 were obtained from the New York State Department of Health (NYDH) and used for THM data (NYDH, 2020). All communities investigated in this study were identified using the U.S. EPA Safe Drinking Water Information System (SDWIS) to link THM concentrations, the served communities, and their demographic information (U.S. EPA, 2022). U.S. Census Bureau's American Community Survey (ACS) 2017-2021 5-Year Estimate (ACS 2021) were used for demographic information such as household incomes and race percentages (US Census Bureau, 2023). We retrieved data for 286 PWS that cover over 80% of the NY state population after excluding very small communities of which information is hard to find on the Census Bureau website. Cancer incidence rate by county was obtained from NY Department of Health (NYDH, 2020). Pearson correlation coefficients (r) were used with corresponding p-values to evaluate the association between THM concentrations and demographic index and between THM concentrations and cancer incidence rates. The significance level of the p-value for determining whether to accept or reject the null hypothesis stating "no correlation between the two variables" was established at 5% (0.05). To compute an EJ Index for THMs, we integrated a normalized THM environmental indicator with the demographic index through the following equation.

$$\text{EJ Index for THMs} = \text{Demographic Index} \times \text{Normalized THM Environmental Indicator}$$

The demographic index was derived from the average of two crucial demographic indicators: the percentage of low-income individuals and the percentage of people of color within the studied population. The percent of people of color represents the proportion of all people other than non-Hispanic white-alone individuals within a community. Meanwhile, the percent of low-income individuals accounts for the share of a community's population residing in households with incomes less than or equal to twice the federal poverty level. The normalization process for the THM environmental indicator involved converting THM data obtained from the New York Department of Health into percentiles in NY state.

Vol. 2024 (1) 31 – 37
ISSN 2688-3651 [online]

3. Results

3.1 Generation of EJ Index for THMs in NY state

Table 1. Communities at or above the 80th percentile of EJ Index for THMs in NY state

Community	Average THM Conc (µg/L)	THM Percentile (NY)	Median Household Income	% Low Income	% People of Color	Demographic Index	EJ Index for THMs	EJ Index for THMs % (NY)
Albany City	54.5	84.5	$48,512	0.414	0.462	0.438	37.0	100
Syracuse City	46.4	73.3	$38,893	0.524	0.466	0.495	36.3	99.7
Potsdam Village	61.9	94.3	$25,850	0.578	0.169	0.374	35.2	99.3
Poughkeepsie City	48.3	76.1	$47,008	0.401	0.514	0.457	34.8	99
Troy City	57.9	90.5	$48,834	0.432	0.337	0.384	34.8	98.6
Rochester City	42.1	63.5	$37,395	0.530	0.546	0.538	34.2	98.3
Peekskill City	47.3	74.3	$66,067	0.296	0.607	0.452	33.6	97.9
Catskill Village	69.1	98.2	$42,200	0.515	0.141	0.328	32.2	97.6
Buffalo City	38.9	58.2	$39,677	0.495	0.529	0.512	29.8	97.2
Geneva City	57.1	88.7	$42,472	0.443	0.223	0.333	29.6	96.9
New York City	41.1	61.4	$70,663	0.347	0.602	0.474	29.1	96.2
Elmsford Village	42.9	65.9	$102,601	0.222	0.629	0.425	28.0	95.8
Monroe Village	63.7	95	$92,744	0.211	0.379	0.295	28.0	95.5
Monticello Village	37.7	52.9	$40,080	0.526	0.528	0.527	27.9	95.1
Port Chester Village	46.1	71.9	$81,586	0.292	0.453	0.373	26.8	94.8
Watertown City	55.8	85.2	$41,918	0.463	0.161	0.312	26.6	94.4
Amsterdam City	45.4	70.8	$40,696	0.467	0.284	0.375	26.6	94.4
Fredonia Village	68.7	97.8	$56,406	0.379	0.159	0.269	26.3	93.7
Schenectady	41.9	62.1	$47,773	0.409	0.435	0.422	26.2	93.4
Wallkill Town	86.9	99.2	$75,825	0.114	0.413	0.264	26.2	93
Newark Village	59.1	91.5	$51,178	0.391	0.176	0.284	25.9	92.7
Oneonta City	66.0	96.1	$55,565	0.392	0.135	0.264	25.3	92
New Paltz Village	50.0	80.7	$75,455	0.457	0.161	0.309	25.0	91.6
Hudson City	38.4	56.1	$36,543	0.528	0.349	0.439	24.6	91.3
Geneseo Village	43.1	66.3	$28,558	0.637	0.104	0.371	24.6	90.9
Utica City	38.2	54.3	$42,624	0.497	0.387	0.442	24.0	90.5
Nyack Village	55.9	85.6	$83,930	0.265	0.291	0.278	23.8	89.8
Lyons Town	56.9	88	$51,354	0.365	0.17	0.268	23.5	89.5
Clarkstown	55.9	85.6	$118,837	0.224	0.324	0.274	23.4	89
Auburn City	48.6	77.5	$43,555	0.416	0.166	0.291	22.5	88.8
Erwin Town	50.0	79.6	$83,773	0.430	0.131	0.280	22.3	88.1
Mount Vernon City	30.9	40.7	$59,291	0.280	0.793	0.536	21.8	87.3
Cortlandt Town	41.2	61.7	$114,347	0.461	0.243	0.352	21.7	86.7
Attica Town	65.4	95.7	$64,093	0.226	0.225	0.226	21.6	86.3
Elmira City	38.4	56.8	$36,543	0.519	0.215	0.367	20.8	86
Fallsburg Town	31.5	41.7	$49,435	0.680	0.306	0.493	20.6	85.3
Syracuse City	31.2	41	$38,893	0.524	0.466	0.495	20.3	85
Rome City	50.5	81.4	$51,752	0.362	0.133	0.247	20.1	84.6
Middle Town City	35.5	47.3	$58,235	0.369	0.473	0.421	19.9	84.3
Binghamton City	36.1	48.7	$35,730	0.536	0.281	0.408	19.9	83.9
Ithaca City	47.3	73.9	$38,019	0.516	0.323	0.419	19.8	83.7
Rensselaer City	56.0	86.3	$56,347	0.258	0.199	0.228	19.7	83.6
New Rochelle City	38.5	57.1	$81,735	0.232	0.45	0.341	19.5	83.2
Medina Village	44.0	68.4	$45,134	0.423	0.134	0.278	19.0	82.9
Seneca Falls	51.9	82.8	$48,961	0.378	0.079	0.229	18.9	82.5
Tupper Lake Village	88.9	100	$42,066	0.300	0.076	0.188	18.8	82.2
Massena Village	57.8	90.1	$45,504	0.352	0.06	0.206	18.6	81.8
Schaghticoke City	73.3	98.5	$94,338	0.321	0.053	0.187	18.4	81.5
Lockport Town	48.6	77.1	$70,060	0.352	0.124	0.238	18.4	81.1
New Paltz Town	39.8	59.2	$75,455	0.457	0.161	0.309	18.3	80.8
Rensselaer city	49.6	78.5	$56,347	0.26	0.199	0.23	17.9	80.1

Vol. 2024 (1) 31 – 37
ISSN 2688-3651 [online]

As U.S. EPA used the 80th percentile as the initial benchmark for addressing environmental justice, we have identified communities in New York State that meet or exceed the 80th percentile of the EJ Index for THMs in the State (Table 1). For example, Albany city had a THM concentration of 54.5 µg/L, which corresponds to the 84.5th percentile in the state. It also had percent people of color of 46.2% and percent low-income of 41.4%, resulting in a Demographic Index of (0.462 + 0.414)/2 = 0.438. An EJ Index for THMs was calculated by multiplying the state percentile for the THM Indicator by the Demographic Index, resulting in 84.5 x 0.438 = 37.0. Once this calculation was applied to all the communities investigated in this study, the state percentile for the THM EJ Index was determined, allowing for easy comparisons among communities. The EJ Index for THMs of 37.0 for Albany city corresponds to the 100th percentile in the state. When a city (e.g. Attica city, Ithaca city) was served by multiple PWS, a population weighted average THM concentration was used. Because the EJ Index combines three measures (THM level, % low income, and % people of color), the percentile of Albany city's THM EJ Index is greater than that of Potsdam village although Albany city's THM concentration is lower than that of Potsdam village. The population weighted average THM concentration for 286 THM data points in New York state in 2020 was 34.2 µg/L, with a standard deviation of 21.4 µg/L. We conducted a regression analysis to evaluate the association between demographic index and THM concentrations but could not find a linear correlation (p-value = 0.49, Figure 1) while a negative correlation was found between median household income and THM concentration in the previous study (Lee and Park, 2023).

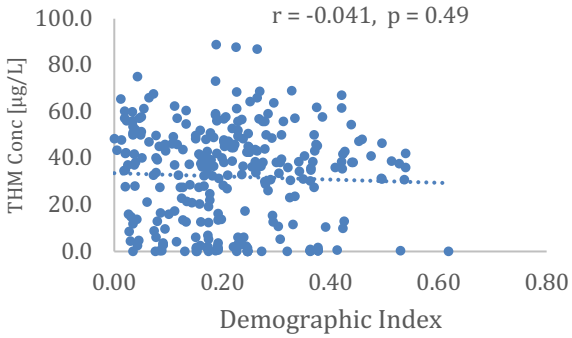

Figure 1. THM concentrations plotted against demographic index at city levels in New York State. THM Conc denotes total trihalomethane concentrations.

Table 2. Communities violated total trihalomethanes standard in NY state in 2020. * denotes cities that violated THM standards multiple times.

County	NY PWS ID	Community	Population	Demographic Index (Percentile in State)
Cayuga	NY0511732	Aurelius (T)*	872	17
Cayuga	NY0530054	Brutus (T)*	1287	6
Cayuga	NY0501718	Fleming (T)	1800	5
Cayuga	NY0501733	Montezuma (T)	490	33
Cayuga	NY0530063	Springport (T)*	52	26
Erie	NY1400397	Akron (V)	3100	45
Essex	NY1500278	Essex (T)*	350	25
Franklin	NY1600012	Tupper Lake (V)*	5500	39
Genesee	NY1800542	Alexander (V)	785	24
Herkimer	NY2102310	Mohawk (V)	2985	41
Jefferson	NY2230022	Champion (T)	650	35
Jefferson	NY2202337	Dexter (V)*	1100	30
Jefferson	NY2202083	Pamelia (T)*	25	45
Jefferson	NY2202346	Watertown City*	27861	54
Jefferson	NY2202352	Watertown (T)*	750	42
Livingston	NY2500701	Caledonia (T)	160	25
Livingston	NY2501014	Leicester (V)*	24	22
Orange	NY3503527	Florida (V)*	2884	47
Orange	NY3503584	Wallkill (T)*	18450	63
Otsego	NY3800154	Oneonta City	15779	41
Steuben	NY5000755	Lindley (T)*	39	21
Tompkins	NY5430047	Ulysses (T)*	400	36
Washington	NY5700124	Whitehall (V)*	2800	54
Wyoming	NY6011605	Castile (T)*	440	40

3.2 State-wide THM Compliance Data

We investigated the THM standard violation history of communities in New York State using drinking water standard violation data from NYDH (NYDH, 2020). In addition to the THM violation data, we included population data from the Census Bureau and Demographic Index data from EJScreen, and generated a combined information sheet (Table 2). Among the communities at or above the 80th percentile of the THM EJ Index, three communities

Vol. 2024 (1) 31 – 37
ISSN 2688-3651 [online]

(Watertown City, Wallkill Town, and Oneonta City, highlighted in red in Table 1) were found to violate the THM standard in 2020. Of the communities with THM standard violations, 48% were served by small public water systems (serving 501-3,300 people), and 35% were served by very small public water systems (serving 25-500 people).

3.3 Correlation between THMs and Bladder and Colorectal Cancer Incidence Rate

We conducted an investigation into the correlation between THM concentrations and the incidence rates of bladder and colorectal cancer in the state of New York. As cancer incidence rates are reported at the county level in NY state, we computed correlations between cancer incidence rates and the average THM concentrations within each county, rather than at the city-level. Our findings revealed no significant correlations, with p-values of 0.98 for bladder cancer incidence rates and 0.120 for colorectal cancer incidence rates (Figure 2). In the 2016-2020 period, the average incidence rate for bladder cancer in New York State's counties was 25.4 cases per 100,000 individuals, with a standard deviation of 4.5 cases per 100,000 people. In comparison, colorectal cancer had an average incidence rate of 13.0 cases per 100,000 individuals, with a standard deviation of 4.1 cases per 100,000 people.

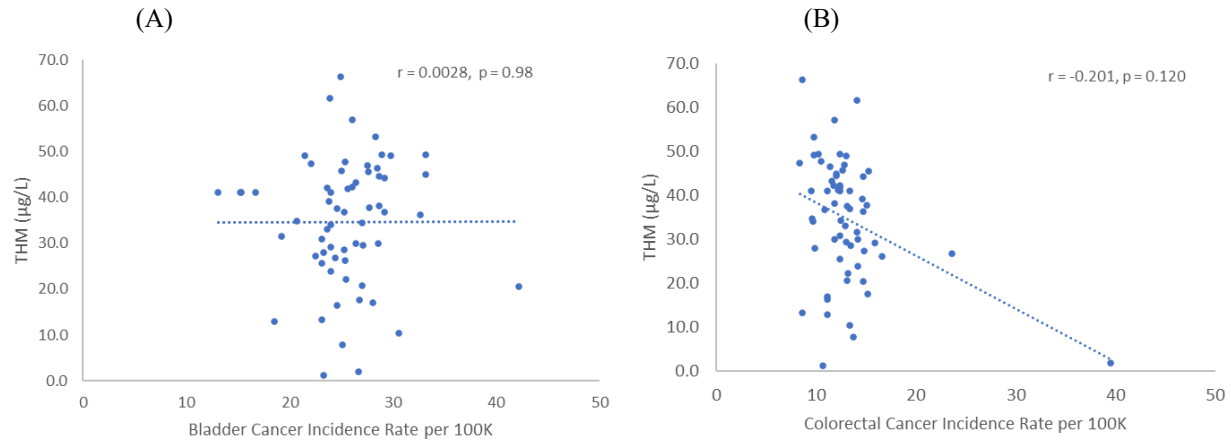

Figure 2. THM concentrations plotted against (A) Bladder cancer incidence rate (B) colorectal cancer incidence rate at county levels in New York State. THM Conc denotes total trihalomethane concentrations.

4. Discussion

Unlike other regulated contaminants in drinking water, DBPs possess unique characteristics as they are by-products formed during a crucial process of inactivating pathogens. They not only form in the final drinking water process at water treatment facilities but continue to form throughout distribution systems. Maintaining low DBPs while ensuring proper pathogen inactivation is a challenging task, contingent on the economic and technical capabilities of communities.

In the U.S. and many other countries, THMs and haloacetic acids (HAA5) are regulated because they serve as indicators of exposure to the complex mixture of DBPs in chlorinated drinking water, although more than 600 DBPs have been identified since the discovery of the first DBPs in 1974. Furthermore, THMs have a more extensive dataset regarding occurrence, carcinogenicity, genotoxicity, and epidemiology than other DBPs, making them suitable for studying correlations with historic socioeconomic factors. Therefore, THMs should be suitable representatives for drinking water quality in EJ studies.

Following a top-down approach recommended by Browne et al. (2022) in a recent EJ study, we developed an EJ Index for THMs in New York State. This process starts with a broad conceptual framework, gradually transitioning to a specific and quantifiable focus, addressing Environmental Justice concerning Drinking Water Quality (Figure 3). In the prior study conducted by Lee and Park (2023), a potential socioeconomic disparity was observed through an examination of the correlation between THM levels in drinking water and median household income in New York

Vol. 2024 (1) 31 – 37
ISSN 2688-3651 [online]

State. To establish a concrete Environmental Justice (EJ) Index, environmental and demographic factors were integrated to encompass the multifaceted dimensions of environmental justice. The final step involves assessing the efficacy of the developed index to ensure that it effectively fulfills its purpose of addressing and rectifying inequalities in access to clean and safe drinking water. This structured approach guarantees a methodical progression from a broad conceptual framework to a practical tool for advocating Environmental Justice.

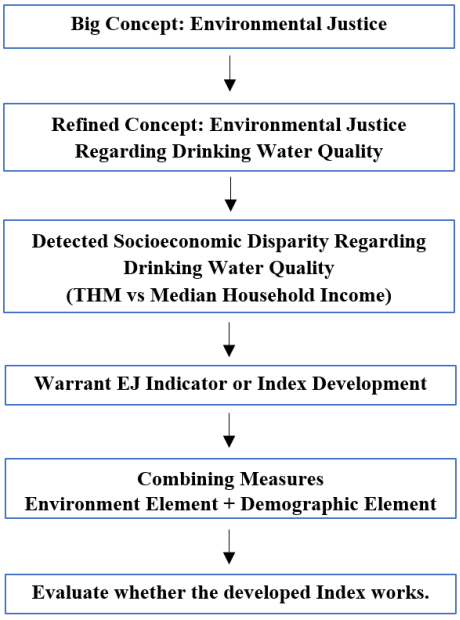

Figure 3. From Concept to Action: Developing an EJ Index for Equitable Drinking Water Quality

Our approach aligns with the guidelines established by the U.S. EPA, which outline key considerations in the selection of environmental indicators, with specific characteristics in mind (U.S. EPA, 2023). First, these indicators should be characterized by a resolution already in existence or readily attainable at the census block group level or a closely related resolution. Second, they should provide broad coverage, with screening level data available or feasible to develop for the entire United States, ensuring comprehensive reach. Third, these indicators should be highly relevant to environmental justice, as they pertain to pollutants or impacts where disparities have been observed between various groups concerning exposures, susceptibility, or health outcomes. Lastly, they should carry significant public health importance, with pollutants or impacts that have the potential for notable effects, raising substantial concerns, even if localized, or demonstrating established links to substantial health impacts on a national scale.

Consequently, we chose THMs as an EJ indicator for several reasons. THM concentrations are typically accessible at the local or regional level through water quality monitoring conducted by various agencies, including state or local environmental agencies. These concentrations are most commonly reported at the community water system level, as community water systems are mandated to monitor and report DBP levels to ensure compliance with federal drinking water regulations, including the total trihalomethanes and haloacetic acids regulations. Nationwide THM concentration data is readily available. As evident in the thirteen EJ Indexes currently utilized by the U.S. EPA (Particulate Matter 2.5, Ozone, Diesel Particulate Matter, Air Toxic Cancer Risk, Air Toxics Respiratory HI, Toxic Releases to Air, Traffic Proximity, Lead Paint, Superfund Proximity, RMP Facility Proximity, Hazardous Waste Proximity, Underground Storage Tanks, Wastewater and Discharge), sets of measurable environmental indicators are essential to comprehend and address unjust exposure to unhealthy environmental conditions. Notably, there is a scarcity of indicators representing water quality, highlighting the need to incorporate more indicators related to water quality.

In the prior study conducted by Lee and Park (2023), an inverse relationship between THM levels and median household income was identified, suggesting a potential socioeconomic disparity in drinking water quality. Elevated DBP concentrations in drinking water have been associated with health concerns, including an increased risk of cancer and other diseases. Communities with higher DBP levels may face health disparities, which can disproportionately affect marginalized or low-income communities. However, we were unable to find such a correlation when we used demographic index instead of median household income for socioeconomic status. While a negative correlation existed between THMs and median household income, there was no observable correlation between THMs and the percentage of people of color. This absence of correlation extends to the demographic index, which incorporates both household income and race factors. The demographic index, comprised of both the percentage of low-income individuals and the percentage of people of color, offers a more robust composite socioeconomic indicator compared to a single metric like median household income or poverty level. This outcome underscores how the definition of demographic factors can profoundly influence the interpretation of environmental justice data in the context of

Vol. 2024 (1) 31 – 37
ISSN 2688-3651 [online]

discussions about environmental justice. The THM EJ Index information included here highlights areas across the NY state that have higher THM levels when compared to other communities in the state but it does not indicate whether a selected area is not meeting the federal or state standard for THMs. For example, the federal or NY state regulatory standard, maximum contaminant level (MCL) of THMs is 80 µg/L and most of communities in the Table 1 did not violate the THM standard.

As a final step of EJ index development, we assessed its effectiveness using state-wide THM compliance data and correlational epidemiology. Although only three communities from the communities at or above the 80th percentile of the THM EJ Index are listed in the THM standard-violating community table (Table 2), it does not mean that only small percentage of the high THM EJ Index communities violated the THM standard. When we developed the EJ Index using 286 data points by matching the names of the communities in the THM data with the geographic names in the U.S. Census Bureau data, we often could not find demographic information for small communities with a population of less than 3,300. Thus, many small communities in Table 2 were not included in 286 data used for the EJ study. However, the retrieved data for 286 PWS covering over 16 million people accounts for 81% of the New York State population, which was adequate for statistical analysis.

We investigated the correlation between THM levels and cancer incidence rates; however, no linear correlations were identified (Figure 2). Access to cancer incidence data at a more granular level, such as the city or village level, would have enabled us to explore potential correlations between the THM EJ Index and cancer incidence rates more comprehensively.

5. Conclusion

Our research has successfully culminated in the creation of an EJ Index for THMs in New York State, employing a meticulously structured top-down methodology in line with established standards. The selection of THMs as a key indicator stems from their data accessibility, relevance to environmental justice, and the significant public health implications they carry. The incorporation of a demographic index underscores the profound influence of demographic factors on the interpretation of environmental justice data. While our EJ Index's efficacy was tested using statewide compliance data, our inquiry into the potential correlation between THM levels and cancer incidence rates revealed the absence of linear associations. To deepen our understanding, access to more granular cancer incidence data at the city or village level is recommended, offering a more comprehensive exploration of possible links between the THM EJ Index and cancer rates. This study underscores the persistent urgency of addressing environmental justice issues and emphasizes the nuanced complexity of interpreting environmental data within the framework of equity and public health. Future research endeavors should encompass the development of THMs and EJ data at smaller scales, such as census block levels, and involve a comparative analysis of the THM EJ Index with other EJ indexes, as presented in the EJScreen.

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Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

Exploring the Antibacterial Potential and Phytochemical Composition of Five Indigenous Indian Medicinal Plants: Effective Strategies Against Multi-Drug Resistant and ESKAPE Pathogens

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Abstract

This paper presents the findings of a secondary research study conducted to investigate the antibacterial potential and phytochemical composition of four indigenous Indian medicinal plants, namely *Salvadora persica* (Miswak), *Caesalpinia pulcherrima* (Peacock flower), *Thymus vulgaris* (Thyme), and *Saussurea lappa* (Kuth). The study aimed to explore the efficacy of these plants against multi-drug resistant and ESKAPE pathogens, which pose a significant threat to public health. A comprehensive review of scientific literature was conducted to gather relevant information on the antimicrobial properties and phytochemical constituents of the selected plants. The antibacterial activity of the plant extracts was evaluated against a panel of multi-drug resistant bacteria and ESKAPE pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species. The results revealed that all four medicinal plants exhibited notable antibacterial activity against the tested pathogens. *Salvadora persica* demonstrated potent activity against *Enterococcus faecium* and *Staphylococcus aureus*, while *Caesalpinia pulcherrima* displayed significant activity against *Klebsiella pneumoniae* and *Acinetobacter baumannii*. *Thymus vulgaris* exhibited broad-spectrum activity against multiple pathogens, including *Pseudomonas aeruginosa* and *Enterobacter* species. *Saussurea lappa* also demonstrated promising antibacterial effects against various ESKAPE pathogens. Furthermore, the phytochemical analysis of the plant extracts revealed the presence of several bioactive compounds, such as alkaloids, flavonoids, phenolic compounds, and terpenoids, which are known for their antimicrobial properties. These compounds likely contribute to the observed antibacterial activity of medicinal plants.

Keywords: ESKAPE, Medicinal plants, Antimicrobial activity, Antibiotic resistance, Bioactive compounds, Alternative medicines, Multidrug-resistant

1. Introduction

The discovery of the first antibiotic - penicillin - was a revolutionary step in medical sciences, providing a dramatically new approach to infection control and healthcare (Bud, 2007). However, the misuse of these drugs has led to antibiotic resistance. Antibiotic resistance occurs when bacteria change in response to the use of these medications, a challenge that is now associated with high morbidity and mortality due to diseases that were otherwise easily treated (Frieri et al., 2017). Antibiotic resistance has been increasingly prevalent wherever these medications are used for humans and animals without prescription. Further, with the lack of standard treatment guidelines in some countries, resistance is becoming increasingly emergent due to overprescription by doctors and veterinarians as well as misuse by patients (Bud, 2007).

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

Today, the rapid development of multi-drug resistant (MDR) pathogens poses a significant challenge to the treatment of infectious diseases, which could grow as threatening as during the pre-antibiotic era (Brown & Wright, 2016). The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*) are characterized by resistance to multiple first-line and last-resort antibiotics (Denissen et al., 2022). The known antibiotic resistance mechanisms of these bacteria include drug inactivation or alteration, modification of drug binding sites, reduced intracellular drug accumulation, and biofilm formation (Santajit & Indrawattana, 2016). These bacteria are recognized by the World Health Organisation as a significant danger to human health (Oliveira et al., 2020). Responsible for nosocomial infections – those that develop while a person is receiving medical attention - they place a significant burden on the health care system and economies. They are associated with high morbidity and mortality, increased treatment costs, diagnostic uncertainties, and distrust in traditional medicines (Santajit & Indrawattana, 2016).

However, medicinal may be a solution to antibiotic resistant. For centuries, Ayurveda has been utilized in India as a treatment for various ailments and illnesses and has popularised the therapeutic uses of plants, initially in the form of crude extracts or powders and further as purified forms (Anand et al., 2019; Khare et al., 2021). Extracts of such plants have been proven to inhibit the growth of pathogens and have no or least toxicity to host cells, opening avenues for the discovery of novel drugs with antibacterial effects. The availability of such alternative medicine and its potential as a solution in a post-antibiotic era necessitates further research and reviews regarding various ayurvedic plants and their interactions.

Although primary research on the antibacterial action of Indian medicinal plants against MDRs has been conducted, there is no present compiled review of their efficacy against the ESKAPE pathogens specifically. Additionally, the secondary metabolites produced by these plants are of particular interest due to their antibacterial properties, both in complex mixtures and as a single active component in their purified forms (Anand et al., 2019; Khare et al., 2021). However, they have not been evaluated across various studies, meaning they cannot be isolated for use in drug development.

To remedy this research gap and explore Indian ayurvedic plants as a solution to the aforementioned socioeconomic problems, a secondary review of four Indian medicinal plants - *Salvadora persica* (Miswak), *Caesalpinia pulcherrima* (Peacock flower), *Thymus vulgaris* (Thyme), and *Saussurea lappa* (Kuth) – has been compiled. This paper aims to discuss the morphology, common uses, antibacterial action and phytochemistry of these plants and compare them.

2. Exploring Indigenous Medicinal Plants

2.1 Salvadora persica

Section 1: Morphology and Common Uses

Salvadora persica, known as miswak or kharijal, is commonly found in the arid regions of India and often in saline soils. It is an evergreen small tree or large shrub, rarely reaching more than 1 ft in diameter and 3m in height ref. It has a soft, white-yellow wood with barks of old stem rugose and numerous drooping branches. It possesses a pleasant small, of cress or mustard, and a warm and pungent taste. The tree produces flowers greenish-yellow in colour and clusters of small, red edible fruits that are juicy but pungent (Khatak et al., 2010).

Research has reported multiple biological effects of *S. persica* including antibacterial, antiviral, antifungal, antibiofilm and antioxidant ones associated with its phytochemistry. (Aljarbou et al., 2022). Due to its use as natural toothbrush for teeth cleaning, literature reviews regarding its dental uses are ubiquitous. However, there is limited and scattered information as to its action against antibiotic-resistant bacteria. To fill this research gap, secondary research and data regarding its efficacy against the ESKAPE pathogens was compiled.

Section 2: Antibacterial Actions of S. persica

Al-Ayed et al., 2016 demonstrated the antimicrobial effects of this medicinal plants against the clinical isolates of 10 MDR pathogens, amongst which were four ESKAPE pathogens: methicillin-resistant *Staphylococcus aureus*,

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

Klebsiella pneumoniae, *Pseudomonas aeruginosa,* and A*ctineobacter baumannii*. The antimicrobial testing of miswak was carried out using the agar diffusion and minimal inhibitory concentration methods (MIC). The study showcased that the methanol extracts of this medicinal plant had greater efficacy than its aqueous extract against tested ESKAPE pathogens due to their greater inhibition zones: 7.6 mm – 13.6 mm for the most effective methanol extract (400 mg/L) as compared to 6.2 mm – 12.0 mm for the most effective aqueous extract., The study recorded the second-highest growth inhibition zone of 12.7 mm against *Klebsiella pneumoniae*. Overall, the authors concluded that *S. persica* is more useful against Gram-negative (3.3 – 13.6 mm) rather than Gram-positive (1.8-8.3 mm) bacteria. Also supported by other studies, this finding is suspected to be due to the different lipo-polysaccharides in their cell membranes and is crucial knowledge for the use of *S. persica* in the development of anti-bacterial medication (Al-Ayed et al., 2016; Alireza et al., 2014).

Alireza et al., 2014 similarly demonstrated the strong antibacterial action of methanol extracts of *S. persica* against the Gram-negative ESKAPE pathogens *Actineobacter baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae* by demonstrating their low MIC values: 0.02, 0.04 and 0.08 mg/L, respectively. In this study, *S. persica* was also found to be more effective than the antibiotics tested against (Kanamycin, Cefotaxim, Fosfomycin, Colistine, Chloramphenicol, and Ticarcillin), widening the avenues for its use in the potential post-antibiotic era.

S. persica methanolic extract was tested and its efficacy was found highest against *P. aeruginosa* and *S. aureus* and relatively lower with *K. pneumoniae*, with inhibition zones of 5.67, 5.00, and 2.00 mm respectively. Not only did this study demonstrate the antimicrobial properties of this medicinal plant against these pathogens but it also did so specifically for wound infections. The study also noted that the greatest incidence of postoperative wound infections in a hospital in Sudan was the result of poor antibiotic selection and increased levels of contamination within the hospital. Thus, the effectiveness of *S. persica* in for wound infections in hospital settings could be extremely useful in lowering mortality rates and reducing economic burden in lieu of failure of antibiotics. (Tatke, 2017).

Section 3: Phytochemical Analysis of S. persica

Al-Ayed et al., 2016 conducted phytochemical analysis of aqueous and methanolic extracts from *S. persica*, which revealed the presence of flavonoids, sterols, saponins, tannins, basic alkaloids and reducing components in alcoholic extracts and saponins, tannins and reducing components in aqueous extract. Notably, the methanolic extract had a wider variety of secondary metabolites that could be responsible for its greater antimicrobial activity in some studies. (Al-Ayed et al., 2016).

Al-Ayed et al., 2016 identified a volatile compound, benzyl isothiocyanate (BITC), in extracts of *S. persica* that showed a strong bactericidal effect against Gram-negative bacteria but only lowered the growth of Gram-positive bacteria. The authors speculated that this phytocompound may penetrate through the outermost membrane of a bacterium, interfere with its redox systems, and hamper its ability to maintain its membrane potential (Al-Ayed et al., 2016).

Authors of (NOR) explored the antimicrobial action and ethnopharmacology of this medicinal plant. They conducted a comparative study on the leaves, tender stems, and tree bark of *S. persica,* discovering that the concentration of BITC is highest (73.5%) in *S. persica* tree bark extract rather than other extracts. Thus, the authors implicated BITC as a potent bacterial inhibitor.

The overall results of these investigations of S. persica indicated that its extracts hold a potential source for plant drugs. They appear most promising not only as a blueprint for antimicrobial drugs but also have several valuable phytochemicals for other ailments. Thus, further research into the phytocompounds present in the tree bark extract and their isolation could propel the development of drugs meant to combat bacterial action.

2.2 Caesalpinia pulcherrima

Section 1: Morphology and Common Uses

Also known as Gulmohar or the peacock flower in India, *Caesalpinia pulcherrima* is a species of flowering plant belonging to the pea family Fabaceae. Usually a shrub or small tree, this plant grows to different heights in different climates. When in climates with few to no frosts, it is only semi-evergreen and can grow up to 3m while in Hawaii, it

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

is evergreen and grows to 5m (Bakshi et al., 1999). It possesses a bi-pinnate foliage with small, oval leaflets and green or greyish-brown stems, depending on how woody they are. After its name, it produces flowers of orange, red, and yellow and is scentless with long stamens and pistils (Bakshi et al., 1999).

Typically, its flowers are harvested for medicinal uses such as to combat intestinal worms and cure sores. West Indians also make decoctions from its leaves and flowers that treat fever. As its leaves have purgative properties, they can be used for abortions and to make decoctions with its flowers to treat fever. Indonesians even pound it to treat children experiencing convulsions (Kumar et al., 2010). A phytochemical and pharmacological review of this plant in Pankal et al., 2014 elucidated its anticancer, antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, immunosuppressive, and vasorelaxation properties (Pankaj et al., 2014). Considering the broad scope of *C. pulcherrima'*s applications worldwide as an ayurvedic plant and its various therapeutic properties, this review regarding its phytoconstituents and efficacy against ESKAPE pathogens was prompted.

Section 2: Antibacterial Action of C. pulcherrima

Parekh & Chanda 2007 tested the antibacterial activity of aqueous and methanolic extracts of the aerial parts of *C. pulcherrima* against three ESKAPE pathogens: *Staphylococcus aureus, Klebsiella pneumoniae, and Enterobacter aerogenes*. While the methanolic *C. pulcherrima* extract inhibited the ESKAPE pathogens, the aqueous extract was only effective against *S. aureus* and *K. pneumoniae*. Overall, the maximum antibacterial activity was exhibited by *C. pulcherrima* out of the 12 medicinal plants tested, with its methanolic extract producing the largest inhibition zones (14 mm – 20 mm). Notably, it was found to be most effective against Gram-negative *K. pneumoniae,* which had an inhibition zone of 20 mm. (Parekh & Chanda, 2007). While Exner et al., 2017 similarly reported that Gram-negative bacteria, *K. pneumoniae*, were most susceptible to this medicinal plant, however, other researchers have opposed this result (Exner et al., 2017). Authors of Swami et al. are of the opinion that the flower parts of *C. pulcherrima* were more effective against Gram-positive than Gram-negative bacteria, attributing this to the presence of an outer membrane possessing hydrophilic polysaccharide chains that forms an additional barrier (Swamy et al., 2014). Similarly, Pulipati et al. also reported that Gram-positive bacteria were more susceptible to *C. pulcherrima*'s flower extract than Gram-negative bacteria (Pulipati et al., 2012). This inconsistency could be due to varying experimental conditions or other extraneous variables and necessitates further research to arrive at a conclusion.

In the study conducted by Refahy et al. various extracts of *C. pulcherrima* were tested against *S. aureus* and *P. aeruginosa*: 100%, 95%, 85%, and 70% methanol, diethyl ether, petroleum ether, methylene chloride, ethyl acetate, and n-butanol. The total phenolic content (TPC) and total flavonoid content (TFC) in these extracts were also measured. It was found that the 70% methanol extract was most effective against *S. aureus*, with an inhibition zone of 10 mm. In comparison, the 100% methanolic one inhibited *P. aeruginosa* the most (10 mm), despite the TPC and TFC being the highest in other concentrations (Refahy et al., 2015). This suggests that flavonoids and phenolics are not the primary phytochemicals responsible for the antibacterial action of *C. pulcherrima.* Notably, the inhibition zones for the other ESKAPE pathogens were between 8-10 mm, indicating that *C. pulcherrima* inhibited them similarly.

Urinary tract infections are those caused anywhere in the urinary tract by microorganisms and are amongst the most common nosocomial infections. Of the 6 ESKAPE pathogens, *S. aureus, E. faecalis, P. aeruginosa,* and *K. pneumoniae* have been implicated as causes of UTIs. While antibiotics are commonly used to treat these infections, with the rise of MDR pathogens, their management, and treatment become more difficult. This occurs especially in hospital settings, where UTIs represent 30-40% of all nosocomial infections (Swamy et al., 2014). Swami et al. explored the efficacy of *C. pulcherrima* extracts against the *S. aureus* and found that they exhibited the strongest inhibition activity against them as compared to other flower extracts tested (*P. ferrugineum* and *D. regia*) (Swamy et al., 2014). This finding is supported by the higher inhibition zones of *C. pulcherrima* (1.4-1.9 cm) as compared to the other flowers (1.1-1.6 mm).

Multiple establishments of *C. pulcherrima*'s inhibition of *S. aureus*, *K. pneumoniae,* and *P. aeruginosa* across various studies indicates that it is an effective antimicrobial agent for drug-resistant as well as drug-susceptible strains of these pathogens.

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

Section 3: Phytochemical Analysis of C. pulcherrima

For the purpose of identification of major phytochemical constituents in a methanolic extract of *C. pulcherrima* flowers, a phytochemical analysis was carried out in Refahy et al., 2015. It revealed the presence of secondary metabolites such as glycosides, flavonoids, phenolics, and tannins (Refahy et al., 2015). JM & Sreeja, 2018 conducted a simlar analysis of another methanol extract of this plant, which indicated the presence of saponins and terpenoids along with the aforementioned phytoconstituents. Additionally, this study once again demonstrated how secondary metabolites found in extracts of this flower differ from solvent to solvent. The methanolic extract consisted of the largest number of phytochemicals, followed by petroleum ether, acetone, and finally ethanol (JM & Sreeja, 2018). As the antibacterial properties of medicinal plants are speculated to be the result of their phytoconstituents, this serves to highlight how crucial the choice of solvent for their extraction is, especially for drug development.

JM & Sreeja 2018, isolated and tested some phenolic compounds from *C. pulcherrima* flower extracts against clinical isolates of some ESKAPE pathogens. Methyl gallate was found to be a potent bactericidal agent against methicillin-resistant *S. aureus* and *K. pneumoniae*. A time-kill kinetics assay of MRSA (methicillin-resistant *Staphylococcus aureus*) using methyl gallate was carried out, showing a reduction in CFU (colony forming units) in two hours and complete bactericidal activity at 24 hours. Methyl gallate was also synergistically tested with Amoxicillin against MRSA and a remarkable fall in MICs (minimum inhibitory concentrations) of both - from 256 µg/ml to 0.0625 µg/ml and 500 µg/ml to 31.25 µg/ml, respectively - was discovered. Along with this compound, gallic acid, and ethyl gallate also exhibited inhibitive properties against the MDR isolates (Khan et al., 2021).

2.3 Thymus vulgaris

Section 1: Morphology and Common Uses

Thymus vulgaris, or common garden thyme, is an aromatic and perennial flowering plant from the Lamiaceae family (Patil et al., 2021). While it originated from Southern Europe, it is now widely cultivated in Indian regions such as the Nilgiris and the foothills of the Himalayas (Hosseinzadeh et al., 2015). It can grow to 6-12 feet tall and its numerous, somewhat woody stems from a foliage mound and are covered in highly aromatic grey-green leaves. Tiny, tubular, and lilac flowers appear at the end of the stems during late spring and early summer (Stahl & Venskutonis, 2012). The plant flourishes well in arid environments and unshaded areas in coarse, rough, and well-drained soils that normally prove hostile to other plants (Javed et al., 2013).

A commonly used plant, *T. vulgaris* possesses a variety of ethnobotanical and culinary applications. It has been used as a treatment for wounds due to its healing and antiseptic properties. The plant has also been reported to possess antiseptic, astringent, carminative, tonic, and anthelmintic properties. Overall, its wide array of properties, especially bactericidal ones, necessitates a thorough review of its potential use against MDR pathogens (Patil et al., 2021).

Section 2: Antibacterial Action of Thymus vulgaris

Qureshi et al., 2022 investigated the antimicrobial, antibiofilm, and antiphage activity of *Thymus vulgaris* extracts against four multidrug-resistant bacteria - *E. coli, S. aureus, C. albicans,* and *P. aeruginosa.* The bioactivity of thyme oil and thyme aqueous extract was investigated, with both showing bactericidal action against these ESKAPE pathogen isolates. However, thyme oil was reported to have greater antimicrobial action than aqueous thyme extracts due to higher zones of inhibition against *S. aureus* and *P. aeruginosa* (49 and 31 mm as compared to 35 and 20 mm, respectively). The authors speculated that this may be due to its higher phenolic content as the aqueous extract loses its active components during grinding and boiling. However, both thyme oil and its aqueous extracts were still found to be most effective against *S. aureus*, with MIC values of 0.2 mg/L and 40 mg/L, respectively. Notably, even though *P. aeruginosa* was found to have the greatest antibiotic resistance out of all isolates, thyme oil showed strong inhibitory action against it even at lower concentrations (0.8 mg/L). Using scanning electron microscopy, the authors researched the effect of thyme on biofilm formation by MDR strains, a previously reported mechanism of antibiotic resistance. Biofilms are microbial communities that adhere to biotic and abiotic surfaces with the cells inside them sheathed in a self-produced matrix. The bacterial cells exposed to thyme were observed to be irregularly shaped, with holes and cracks. Accordingly, it was suggested that thyme treatment induces damage to the cell membrane, eventually causing

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

lysis. These findings are concordant with the results of Helander et al., 1998, which reported the mechanisms of action of thymol (a major active component of thyme) in attacking the outer membranes, resulting in the disruption of the cell wall and a subsequent release of intracellular components and depletion of ATP (Qureshi et al., 2022) (Helander et al., 1998).

Similar effects were also reported by Alibi et al., 2020 wherein along with the antibacterial and anti-biofilm, the anti-quorum sensing (QS) and antioxidant properties of thyme were explored. The intercellular signalling mechanism, known as quorum-sensing, is the process through which antibiotic resistance, biofilm production, and virulence properties are controlled. In this research, forty-four strains of multi-drug resistant bacteria, of which 11 were variants of ESKAPE pathogens, were tested against four essential oils. These 11 strains were of the species *K. pneumoniae*, *S. aureus*, *A. baumannii*, and *P. aeruginosa*. Along with *Cinnamomum verum* and *Eugenia caryophyllata*, the *T. vulgaris* EO (essential oil) demonstrated remarkable antimicrobial action against all tested MDR strains. The MBC/MIC (minimum bactericidal concentration/minimum inhibitory concentration) ratios of these essential oils were equal to 1 for most bacteria, suggesting a bactericide effect. Biofilm formation was observed in all tested strains but a significant reduction in this mechanism was observed due to sub-inhibitory concentrations of thyme essential oil. Finally, the anti-QS activity of the four EOs was reflected in a decrease in violacein production, with the highest being shown by thyme oil at 99.41%. Notably, no significant differences were observed between the bactericidal action of essential oils against Gram-negative versus Gram-positive bacteria in this study (Alibi et al., 2020).

Amorese et al., 2018 studied the activity of essential oils against *P. aeruginosa* isolated from infected hip implants. Periprosthetic joint infections are one of the most severe and costly complications of joint arthroplasty. These infections are also associated with significant psychological and physical morbidity in patients. In this study, the susceptibility of sixteen strains of *P. aeruginosa* against 11 antibiotics and thyme essential oil. All strains showed multiple antibiotic resistance but were susceptible to aminoglycosides, third-generation fluoroquinolones, third-generation cephalosporins, and some carbapenems. Thymus vulgaris was found to be effective on eleven strains of *P. aeruginosa* at 8% concentration and the remaining five variants at 16% (Amorese et al., 2018).

Other studies have also reported the antibacterial action of *T. vulgaris* against ESKAPE pathogens such as *E. faecium* and other strains of *S. aureus* and *P. aeruginosa* (Jain & Choudhary, 2022).

Section 3: Phytochemical Analysis of Thymus vulgaris

Owing to its commonality, various researchers have studied the phytochemical constituents of *Thymus vulgaris* through methods such as gas chromatography-mass spectroscopy (GC-MS) and high-performance liquid chromatography (HPLC). Overall, the presence of phenolic compounds, terpenoids, flavonoids, steroids, alkaloids, tannins, and saponins was detected in the thyme extracts. Notably, volatile compounds were found to be most prevalent (Abdelli et al., 2017; Al-Asmari et al., 2017; Stefanis et al., 2019). A deeper dive into the essential oils of *T. vulgaris* demonstrates the presence of a list of hydrocarbons, oxides, alcohols/esters, and aldehydes/ketones. Among all of the volatile compounds, major pharmacological activities have been attributed to thymol, carvacrol, geraniol, linalool, α- and β-pinene, p-cymene, and γ-terpinene (Patil et al., 2021). Thymol and carvacrol have been specifically and thoroughly explored in relation to their antibacterial effects. The most frequently reported mechanism of both isomers is bacterial lysis, leading to leakage of intracellular components and cell death. Other mechanisms include the inhibition of efflux pumps, prevention or disruption of biofilm formation, decreasing bacterial motility, and inhibition of membrane ATPases (Kachur & Suntres, 2020).

Similarly, specific phenolic compounds, flavonoids, steroids, tannins, alkaloids, saponins, and other phytoconstituents have also been found. Still, as most of the chemical components are classified under phenolic compounds and essential oils, the specific therapeutic mechanisms and potential of other phytochemicals are yet to be explored (Patil et al., 2021). The antimicrobial effect of thyme essential oils depends on incubation, synergistic ingredient effects, the solvent used for extraction, and more. Hossain et al. demonstrated how the phytoconstituents of thyme extract differ from solvent to solvent, with its butanol extract yielding the greatest number of compounds (Hossain et al., 2022).

Overall, despite their proven antibacterial action, there is limited use of thyme essential oils and other phytochemicals in the pharmaceutical industry. Thus, information regarding optimal solvents, extraction conditions,

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

and the synergistic effects of these compounds with each other and antibiotics must be gathered and compiled to enable the application of *T. vulgaris* in the medicinal community.

2.4 Saussurea lappa

Section1: Morphology and Common Uses

Saussurea lappa, better known as kuth or kushta, belongs to the Asteraceae family and is another perennial herb widely used in Ayurveda. In India, it can be found in the Kashmir and Alpine Himalayan ranges at 8,000 to 12,000 feet. It is a robust, erect plant that can grow 25 to 80 cm high. Its roots already have various applications in the pharmaceutical industry and are stout, brownish with longitudinal streaks or furrows and can grow to 60 cm long. They have a distinct, sweet, and pleasant odour but taste bitter. It has simple alternating leaves that are radical, irregularly toothed and have long lobately winged stalks. Dark purple or black flowers occupy its axillary and terminal heads and the plant produces two kinds of fruits - Achenes and Pappas. Pappas is about 1.7 m long, feathery and brown while Achenes is 3 mm long, curved and compressed (Alaagib & Ayoub, 2015; Gautam & Asrani, 2018).

In India, this herb has been mentioned in the Atharvaveda and is regarded as an ancient Vedic plant god. It was supposedly derived from a heavenly plant originating from the Himalayas. It was used to strengthen and stabilize digestion, increase fertility, ease pain, and even as an ointment for ulcer treatments (Tharak & Zuhra, 2012). Research has now confirmed its pharmacological properties, which are in line with its traditional uses. Another secondary review delved deep into the antitumor, antibacterial, anti-inflammatory, hepatoprotective, antiulcer, cholagogic, and immunomodulatory properties. The same paper also reported the various miscellaneous activities of this medicinal plant including cardiovascular, anticonvulsant, antiparasitic effects, and more (Gautam & Asrani, 2018). Keeping its commonality and a wide array of applications in mind, the following review was compiled to assess its effectiveness against ESKAPE pathogens.

Section 2: Antibacterial Action of Saussurea lappa

Naseer et al. 2022 evaluated the antibacterial effects of *Saussurea lappa* against the multidrug-resistant human pathogenic bacteria *K. pneumoniae*, methicillin-resistant *S. aureus*, *P. aeruginosa*, *E. coli* and Extended Spectrum Beta-Lactamase (ESBL). Using the agar well diffusion method, the authors assessed the dose-dependent antimicrobial effects of the ethanolic *S. lappa extract*. The plant was found to be effective against the four aforementioned ESKAPE pathogens and its ethanol extract exhibited a concentration-dependent zone of inhibition with the largest zone of inhibition corresponding to the highest concentration and *vice versa*. The effect of *S. lappa* varied from pathogen-to-pathogen. At a concentration of 2000 µg/mL, its ethanol extract was bacteriostatic against *S. aureus* and *P. aeruginosa* but effective against *K. pneumoniae and A. baumannii.* However, the herb extract turned bactericidal against *S. aureus* and *P. aeruginosa* at concentrations of 6000 µg/mL and 4000 µg/mL, respectively.

Another research explored the effectiveness of different *S. lappa* extracts against six ATCC (American Type Culture Collection) and three MDR strains: *S. aureus*, *K. pneumoniae* and *P. aeruginosa.* The roots, leaves, stems, and flowers of *S. lappa* were extracted using methanol and chloroform solvents. Their bactericidal activities were evaluated using the agar well diffusion method. The results of this study revealed the potent antibacterial action of the plant extracts against the ATCC and MDR strains. Overall, the greatest effect was observed for the chloroform leaf extract against MDR *P. aeruginosa*, which had the highest inhibition zone of 21 mm. While all solvent extracts inhibited the activity of MDR *K. pneumoniae*, the methanolic stem extract was ineffective against MDR *P. aeruginosa* and all methanolic extracts were bacteriostatic to the *S. aureus* strains. Additionally, the results showed that the leaf and root extracts of this plant were most inhibitory against the bacteria, especially the MDR ones (Naseer et al., 2022) as they had the highest inhibition zones and lowest MICs.

The strong antimicrobial of *S. lappa* has also been reported in Omer et al. 2019 where the effect of its ethanolic and aqueous root extracts against *S. aureus* and *Salmonella sp.* The water and ethanol extracts were only bactericidal against *S. aureus*. Further, the ethanolic extract was generally more effective than the aqueous one with a mean inhibition zone of between 18 mm to 20 mm as compared to the water extract's 15 mm (Omer et al., 2019).

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

In fact, the roots of this plant seem to possess potent antimicrobial activity as their methanolic, cold water, and hot water extracts showed inhibitory effects against *S. aureus, P aeruginosa,* and *E. faecalis* (Khalid et al., 2011). Overall, the methanolic extract exhibited the greatest bactericidal action with a maximum inhibition zone of 18 mm against *P. aeruginosa* and *S. aureus.* Only the hot water and methanolic extracts managed to inhibit all strains, while the cold water one showed no activity against *E. faecalis* (Khalid et al., 2011).

Both previously mentioned researches attributed these trends to the presence of greater numbers of bioactive compounds in specific extracts, making the consideration of the solvent extraction method crucial for the use of this plant in pharmaceuticals.

Section 3: Phytochemical Analysis of S. lappa

Hasson et al., 2013 accompanied their assessment of *S. lappa's* microbial action against MDR bacteria with a thorough phytochemical review meant to detect the presence of 20 bioactive compounds. Overall, sixteen active components were reported across the chloroform and methanol root, leaf, stem, and flower extracts. The presence of flavonoids, alkaloids, proteins, β-carotene, coumarins, saponins, carbohydrates, fats, and quinones was confirmed in all extracts while glycosides, emodins, and phlorotannins were found in none.

The greatest number of phytoconstituents were found in the methanol root extract at 14, followed by 13 in the chloroform root and leaf extracts. Hasson et al., 2013 attributed the high effectiveness of the methanol root extract of *S. lappa* to the bioactive compound richness and possible synergistic action of two or more compounds against *S. aureus*. Andrographolide, 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, and isosteviol methyl ester are known for their strong pharmacological activities and were present exclusively in ethanol extracts. The potential of andrographolide as a pharmaceutical agent has also been confirmed by Banerjee et al. 2017, which showed how an *S. aureus* strain was found to be sensitive to this compound at a MIC of 100 µg/ml, which is comparable to the amount detected in the ethanol extract (Hasson et al., 2013).

This same research also briefly mentioned the role of fatty acid methyl esters and sesquiterpenes in defence against their microorganisms. Similarly, other studies have also reported the bioactive potential of sesquiterpenes such as costunolide, Isodihydrocostunolide, cynaropicrin in drug development.

3. Conclusion

The plants explored in this secondary review have diverse morphologies but possess biological activities, necessitating this in-depth report on their antibacterial ones.

This study highlights the significant antibacterial potential of *Salvadora persica, Caesalpinia pulcherrima, Thymus vulgaris,* and *Saussurea lappa* against multi-drug resistant and ESKAPE pathogens. The presence of various bioactive phytochemicals further supports their therapeutic potential. A tabular summary and comparison of the activities of these plants can be found below.

Table 3.1: Comparison of the antibacterial effects and phytochemistry of *Salvadora persica, Caesalpinia pulcherrima, Thymus vulgaris and Saussurea lappa*

	S. persica	*C. pulcherrima*	*T. vulgaris*	*S. lappa*
ESKAPE pathogens inhibited	*Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Actineobacter baumannii.* and *Enterobacter cloacae*	*Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter aerogenes, and Pseudomonas aeruginosa.*	*Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter aerogenes, and Pseudomonas aeruginosa.*	*Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa.*

Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

Effective extracts	Methanol extracts are more effective than aqueous ones, with their own activity being highest against *K. pneumoniae* followed by MRSA and *A. baumanii*	Flower extracts of this plant exhibited the strongest inhibition activity against *S. aureus, E. faecalis, P. aeruginosa, and K. pneumoniae.* as compared to others.	Thyme oil proved most effective against MRSA. It was shown to reduce biofilm formation and cause damage to bacterial cell membranes.	Its leaf extracts have been found most effective against MDRs. Some extracts (stem or aqueous ones) are only bactericidal. Roots have potent activity, with the methanol extract proving most effective.
Notable antibacterial action	Shown to be more effective against Gram negative than Gram positive bacteria due to the different lipo-polysaccharides in their cell membranes.	Flower part considered to be more effective against Gram positive than Gram negative bacteria.	Thyme oil also showed anti-quorum sensing properties against MDR strains of K. pneumoniae, S. aureus, A. baumannii, and P. aeruginosa	This is concordant with phytochemical studies, which reported the greatest number of bioactive compounds in methanolic extracts.
Phytochemicals found	Presence of flavonoids, sterols, saponins, tannins, basic alkaloids and reducing components detected.	Presence of glycosides, flavonoids, phenolics, saponins, terpenoids and tannins detected.	The presence of phenolic compounds, terpenoids, flavonoids, steroids, alkaloids, tannins, and saponins was detected in the thyme extracts.	The presence of flavonoids, alkaloids, proteins, β-carotene, coumarins, saponins, carbohydrates, fats, and quinones was detected.
Notable phytochemicals	Benzyl isothiocyanate showed strong bactericidal effects against Gram-negative bacteria but only lowered the growth of Gram-positive bacteria.	A phenolic compound - methyl gallate - was found to be a potent bactericidal agent against MRSA and *K. pneumoniae.* It was symbiotically tested with Amoxicillin and showed significant results.	Thymol and cavarcol have been especially implicated in this plant's antibacterial action, with their main mechanism suspected to be bacterial lysis.	Andrographolide, 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, and isosteviol methyl ester are known for their strong pharmacological activities and were present in the extracts of this plant

These findings provide valuable insights for the development of alternative and effective strategies to combat drug-resistant bacterial infections using indigenous Indian medicinal plants. Further research and clinical studies are warranted to explore their application in the development of novel antimicrobial agents or adjunct therapies.

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Vol. 2024 (1) 38 – 49
ISSN 2688-3651 [online]

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Vol. 2024 (1) 50 – 55
ISSN 2688-3651 [online]

Inhibition of the p53 Oncogene by Extracted Curcumin through Zebrafish Embryonic Models of Cancer

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Abstract

As cancer cells grow uncontrollably and spread to other parts of the body, partly due to the mutation of the p53 proto-oncogene. The mutated p53 proto-oncogene, also known as the p53 oncogene, can no longer regulate cell cycles, allowing for cells with damaged DNA to proliferate. The p53 oncogene can be inhibited by different extracted concentrations of curcumin on zebrafish embryonic models of cancer, as observed through mutations in developing embryos. Zebrafish embryos are an excellent in-vivo modeling tool because 84 percent of the genes known to be associated with human diseases have a zebrafish counterpart. Zebrafish are useful to understand the dynamics of early-stage cancers, especially since they overexpress the p53 oncogene; when this is suppressed, gross mutations of the embryo result. Data for each concentration of curcumin was graded 72 hours post fertilization (hpf) by the severity of several phenotypes (bent or hook-like tails, spinal column curving, sac mutation (reabsorption), shorter body length, or no mutations). Results of the experiment showed that increased concentrations of curcumin, which acts as an anticancer agent, led to more severe mutations, indicating a higher absorbance of curcumin by cancer cells, thus suppressing the overexpressed p53 oncogene.

Keywords: Curcumin, Danio rerio, Cancer, p53 oncogene, p53 proto-oncogene

1. Introduction

Cancer is the second leading cause of death worldwide, as the cancer cells grow uncontrollably and spread to other parts of the body (Cabezas-Sáinz et al., 1978). As researchers continue to find ways to better understand the disease and improve the treatments, they have been working with zebrafish (*Danio rerio*) embryos, a tropical fish native to southeast Asia, as models of cancer. 84 percent of the genes known to be associated with human diseases have a zebrafish counterpart and the biology of cancer is very much the same in zebrafish and humans, based on the genome sequence (Gilbert, 2016). Zebrafish are an excellent model (Khan and Alhewairini, 2018). Due to their cost-effective maintenance, high fecundity, fast development, and the transparency of the embryos which helps visualize tumor cell growth and the dynamics at early stages of cancer. Just like humans, zebrafish have a p53 gene that regulates their cell cycle. p53 acts as a proto-oncogene, and when it mutates, it leads to the mutation of the cell and metastasis to others (Wang et al., 2021). In zebrafish embryos, the same thing occurs with cell death, and as one cell dies, others around it also do so. Research has also been done on curcumin, (diferuloylmethane), the active ingredient of turmeric, which gives turmeric its bright yellow characteristics, has shown anti-cancer effects on cancer cells. Turmeric, a plant in the ginger family, is grown throughout India, other parts of Asia, and Central America (O'Brien, 2018). The therapeutic applications of turmeric (its rhizome, the underground stem) have a long history and are used in culinary and traditional Asian medicines. The objective of this experiment was to investigate if active ingredients in natural ingredients, such as turmeric, could be used during combination therapy to treat cancer patients. Based on the results, the increased concentration of extracted curcumin has implications for significantly inhibiting the p53 oncogene, as displayed through the severe mutations in the zebrafish embryos.

Vol. 2024 (1) 50 – 55
ISSN 2688-3651 [online]

2. Materials and Methods

2.1 Curcumin Extraction:

1 g, 2.5 g, 5.0 g and 10.0 g of turmeric were weighed using a digital scale and were placed into test tubes labeled with weights to extract the concentrated turmeric. Laboratory grade acetone, bought from Carolina Biologicals (North Carolina, USA), was used as a solvent. Using a 10 mL graduated cylinder, acetone was poured into each of the four test tubes labeled with the turmeric weight and 10 mL of distilled water was poured into one test tube labeled as control. The test tubes with turmeric were stirred for about 5 minutes separately until the turmeric in the test tubes became a homogeneous slurry. The slurry was filtered out using filter paper and poured into test tubes labeled with turmeric mass. The standard curve was calculated using a spectrophotometer for the different weights of turmeric, using 6 glass cuvettes of which 4 were labeled with turmeric mass, one as control (water) and one as acetone to act as a blank. 1 mL of each filtrate from the test tubes were pipetted into the labeled cuvettes to observe the absorption of photons which was then recorded. The filtrate from the cuvettes was then placed back into the original test tubes respectively and the filtrate was then let to evaporate ensuring acetone was completely evaporated. After the filtrate is complete evaporated 1 mL of acetone was poured into each of the test tubes and the contents were stirred using a glass stirrer separately and using different pipettes, 1 mL of the mixture was placed into the weight labeled petri dishes, and 1 mL of distilled water was placed into the control petri dish. Negative absorbance defines the concept that less light can pass through the extracted substance, which correlates to a greater amount of curcumin being extracted. The sensor used was the GoDirect SpectroVis Plus Spectrophotometer with a wavelength range of 380 nm to 950 nm, a wavelength accuracy of ± 4.0 nm, and a photometric accuracy of ± 0.10 AU. The blank, which was different from the control, was used to calibrate the spectrophotometer and was not further used in data analysis. The regression line in Figure 1 implies that as the amount of turmeric used increased the concentration of curcumin increased.

Table 1: Absorbance Values Based on Mass of Turmeric Used for Curcumin Filtrate.

Substance (mg)	Absorbance (AU)
0.0	0.00887
1.0	0.00166
2.5	-0.00101
5.0	-0.00605
10.0	-0.01

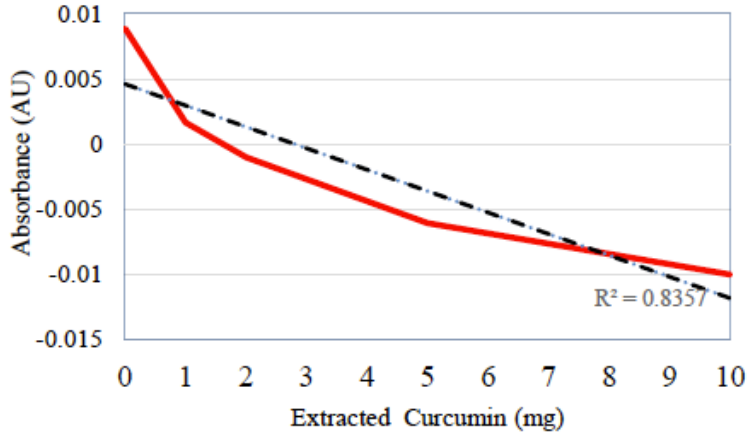

Figure 1: Standard Curve Absorbance Slope Based on Extracted Curcumin from Different Masses of Turmeric

2.2 Zebrafish Breeding and Eggs:

The male and female zebrafish were bought from Carolina Biologicals and were placed into two separate tanks. The tanks were set up with 30 liters of water and included a filter system and automatic food feeder, and the water temperature was maintained at 22 ^{0}C with automatic lights turned off at night. The fish were let to get acclimated to their environment for 3 days. On the 4th day, a breeding tank bought from Carolina Biologicals was used to breed the fish. Water from both the male and female fish tanks was used; two male fish and one female fish were placed into the breeding tank with the divider for the fish to get acclimated. After one and half hours the divider was removed to let the fish mate naturally. After 2.5 hours later when the eggs were collected at the bottom of the breeding tank, the male and female fishes were placed back into their respective tanks. Water from the breeding tank was placed in the

Vol. 2024 (1) 50 – 55
ISSN 2688-3651 [online]

petri dishes. Five eggs were carefully pipetted one at time into the turmeric weight labeled petri dishes and into the control petri dish with water. The petri dishes were then placed in a warm area and let the eggs develop.

2.3 Data Recording and Disposal:

At 24 hours post fertilization (hpf), curcumin was introduced to the embryos. The mutations (Wu et al., 2007) were observed from 48- 72 hpf and the phenotype data was recorded. Severity score defines the qualitative observation of how much the extracted curcumin at differing concentrations has affected zebrafish development by inhibiting the p53 oncogene. Mild indicates that the zebrafish embryo is following normal development markers, while moderate refers to more obvious mutations and severe defines embryos that have been mutated to the point that development has stopped. Within 168 hours of post fertilization, all embryos except in the distilled water control group were euthanized by the supervisor in a two-step process involving freezing and bleach. Embryos in the distilled water control group (with no abnormalities during development) were placed into a fish tank to develop into adults. The above process breeding and observing mutation was conducted for two more trials.

3. Results

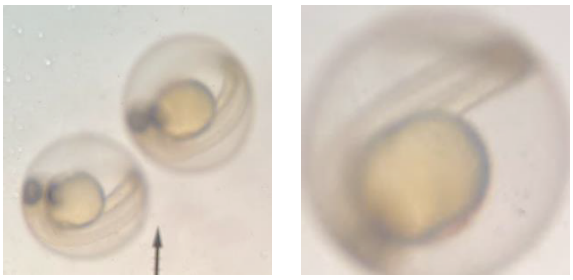

Figure 2: Control group embryonic development (exposed to only distilled water) at 72 hpf was observed to have normal expected zebrafish development.

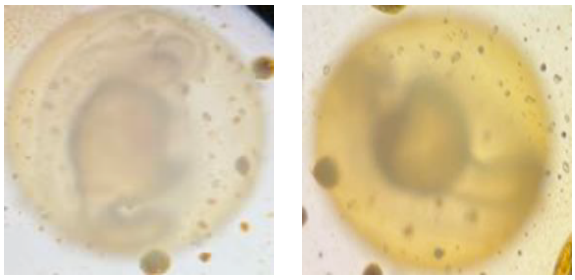

Figure 3: The 1 g group's embryonic development at 72 hpf showed slight mutations in tail development, spinal column curving, body length, and sac reabsorption. Phenotypes were comparable to the control group.

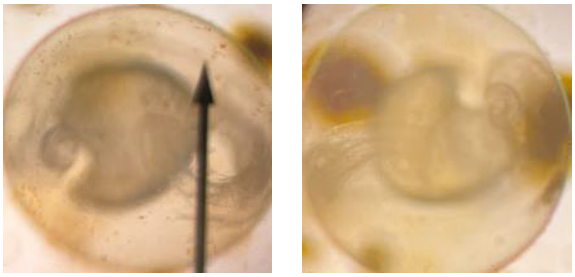

Figure 4: The 2.5 g group's embryonic development at 72 hpf showed some mutations in tail development, spinal column curving, body length, and sac reabsorption. Phenotypes were comparable to 1 g embryos and the control group.

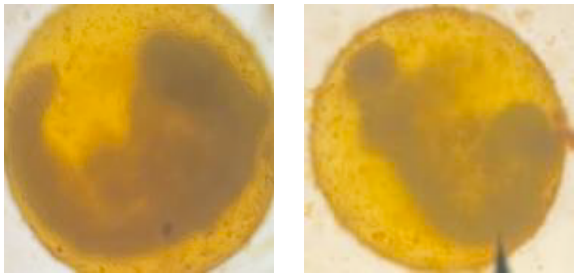

Figure 5: The 5 g group's embryonic development at 72 hpf showed moderate to severe mutations in tail development, spinal column curving, body length, and sac reabsorption.

Vol. 2024 (1) 50 – 55
ISSN 2688-3651 [online]

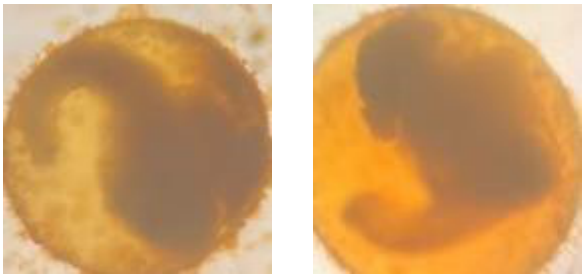

Figure 6. The 10 g group's embryonic development at 72 hpf showed extremely severe mutations in tail development, spinal column curving, body length, and sac reabsorption.

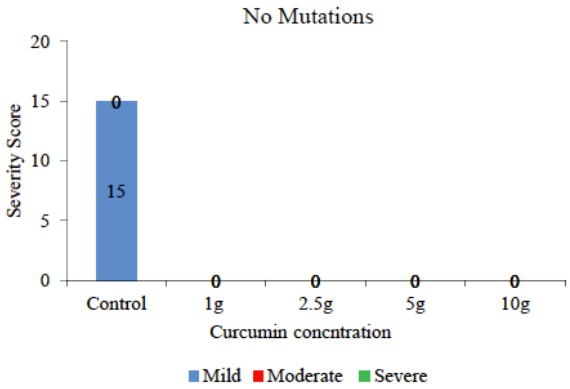

Figure 7. Graph of groups that received no mutations (only control).

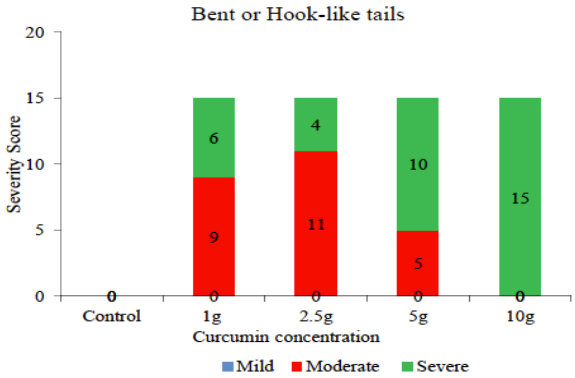

Figure 8. Severity (on a scale of mild to moderate to severe) of bent or hook-like tail mutations.

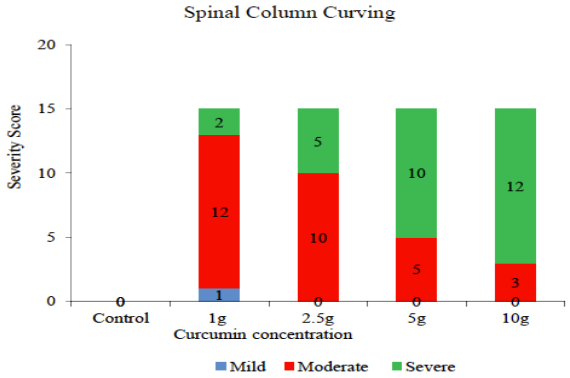

Figure 9. Severity (on a scale of mild to moderate to severe) of spinal column curving.

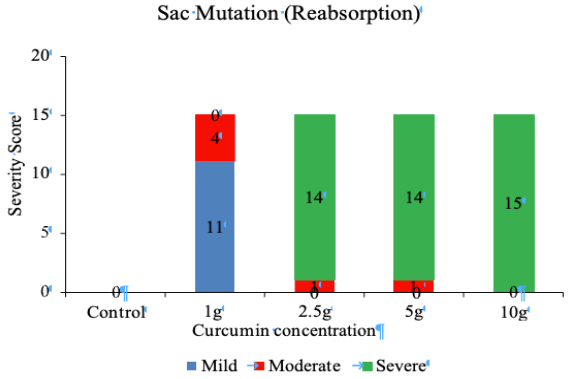

Figure 10: Severity (on a scale of mild to moderate to severe) of sac mutations (sac reabsorption)

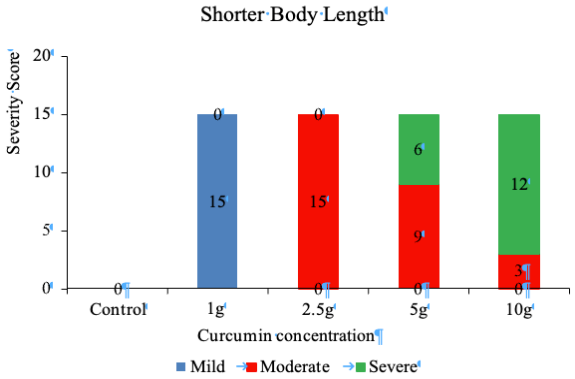

Figure 11. Severity (on a scale of mild to moderate to severe) of shorter body length mutations.

4. Discussion

A standard curve was used to determine the exact concentration of curcumin from acetone through the measure of absorbance. As a blank, acetone was used with an absorbance of 0.00887 AU. Smaller measurements were used and then scaled to fit the spectrophotometer. 1.0 mg had an absorbance of 0.00166 AU, 2.5 mg had a level of -0.00101

Vol. 2024 (1) 50 – 55
ISSN 2688-3651 [online]

AU, 5.0 mg had an absorbance level of -0.00605 AU, and 10 mg had an absorbance of -0.01 AU. The decreasing values can be seen on the standard curve. The control group, where there were just eggs with water, there were 15 eggs with no mutations. For 1 gram of concentrated curcumin, there were 9 with moderate bent or hook-like tails, and 6 with severe bent or hook-like tails. There were also 1 mild, 12 moderate, and 2 severe embryos with spinal column curving. 1 g of concentrated curcumin also had 11 mild and 4 moderate mutations of sac reabsorption. All 15 showed mild mutations of shorter body length. For 2.5 g of concentrated curcumin, 11 embryos had moderate bent or hook-like tails and 4 with severe mutations. 10 had moderate spinal column curving and 5 were severe. There was 1 moderate sac reabsorption and 14 severe mutations. All 15 showed mild shorter body length. For 5 g of concentrated curcumin, there were 5 with moderate bent or hook-like tails, and 10 with severe bent or hook-like tails. There were also 5 moderate, and 10 severe embryos with spinal column curving. 5 g also had 1 moderate and 14 severe mutations of sac reabsorption. 9 showed moderate mutations and 6 showed severe mutations of shorter body length. For 10 g of concentrated curcumin, all 15 embryos had severe bent or hook-like tail mutations. 3 had moderate spinal column curving and 12 were severe. All 15 showed severe mutations. 3 showed moderate shorter body length and 12 showed severe body length mutations.

The objective of this experiment was to observe the effect of the concentration of curcumin extracted by acetone on its anti-tumor properties, as observed through zebrafish eggs as a model of cancer. The hypothesis was that if the concentration of the curcumin extracted from turmeric is increased, then the extracted substance will have higher anti-cancer properties. The hypothesis was supported due to the increased concentration of turmeric used in the acetone extraction process, causing more curcumin to be extracted. All trials showed some signs of mutation properties compared to water. However, there were sources of error. Some of the pipetting may have not been exact, so the exact concentration of curcumin per dish may have varied slightly. Additionally, because all embryos came from the same parents, there was very little genetic variation, which must be considered as cancer affects those with certain DNA variations differently.

A future direction could be to consider sex differences into account by using full grown fish, as gender in healthcare leads to prominent reactions to the same treatment. Mutations in females could respond differently to curcumin than those in males, and the concentration of curcumin may work more efficiently on one sex than the other. Another area of improvement for the project would be to test curcumin on actual tumor cells to observe its effect on the rate of cell division and metastasis, because although zebrafish embryos are a good model of cancer in humans, cancer cells have other mutations present which may cause them to respond differently to treatment.

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