



Formulation and Evaluation of Polyherbal Mouthwash

Lovish Jasrotia¹, Sanchit Dhankhar^{1*}, Nitika Garg¹¹Ganpati Institute of Pharmacy, Bilaspur- 135102, Haryana, India

ARTICLE INFO

Keywords:

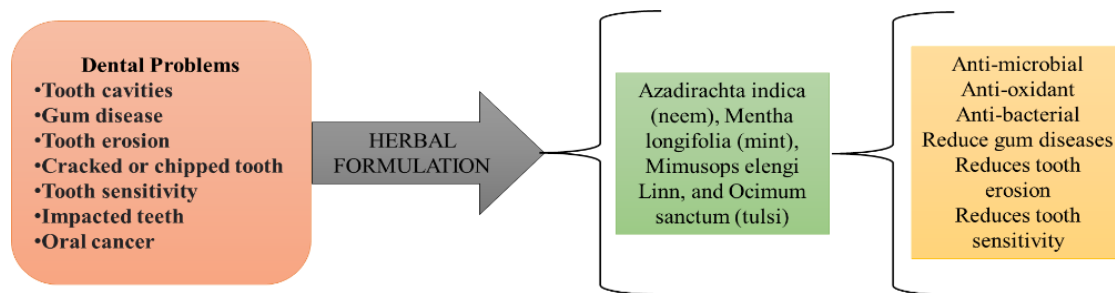
Antioxidants,
Herbal extracts,
Oral health,
Mouthwash,
Azadirachta indica,
Mentha longifolia,
Mimusops elengi Linn.

Abbreviations:

ME: *Mimusops elengi*;
GAE: Gallic acid equivalent;
NO: Nitric oxide;
OS: *Ocimum sanctum*;
ML: *Mentha longifolia*;
AI: *Azadirachta indica*;
ROS: Reactive oxygen species.

ABSTRACT

The current research intends to form a mouthwash from four herbs—*Azadirachta indica* (neem), *Mentha longifolia* (mint), *Mimusops elengi* Linn, and *Ocimum sanctum* (tulsi)—and to evaluate the efficacy of these herbs as an antioxidant herbal mouthwash. Dental caries and periodontal disease are the most common infectious illnesses, and they may be caused by a large number of pathogens and microorganisms at different times during the life cycle of the host. Current mouthwash is a liquid formulation that, based on the active components, may be employed to relieve oral irritation and infection. The Ayurvedic mouthwash was formulated by employing *Azadirachta indica* (neem), *Mentha longifolia* (mint), *Mimusops elengi* Linn, and *Ocimum sanctum* (tulsi), and the phytochemical and antioxidant analysis of these has been conducted. The findings indicated that these herbs possess strong antioxidant capacity and could serve as a good herbal mouthwash formula.



Graphical abstract: A detailed study on the Development and Effectiveness of Herbal Mouthwash

Introduction

Oral health is a critical part of general well-being, which in turn has an influence on many dimensions of everyday life such as speech, nutrition, and socialisation [1-8]. Cell damage and tissue dysfunction are the consequences of oxidative stress, in which the body's antioxidant defense mechanisms are overpowered by the body's production of reactive oxygen species (ROS) [9-11]. Such oxidative damage has been implicated in a range of oral health issues, including periodontal diseases, dental caries, and oral

mucosal disorders [12-15]. Given this, the importance of antioxidants in oral health has grown, with possible benefit in the prevention and control of a variety of oral health conditions [16-18]. Herbal medicine, with its strong roots in conventional knowledge and practices, provides an abundance of possible sources for natural antioxidants that can be exploited to remedy oral health issues [19-24]. Among the myriad of herbs, *Azadirachta indica* (neem), *Mentha longifolia* (mint), *Mimusops elengi* Linn, and *Ocimum sanctum* (tulsi) have been exceptional candidates because of their use in traditional

* Corresponding author E-mail address: sanchitdhankhar@gmail.com (Lovish Jasrotia).

systems of medicine and reported antioxidant activity [25-34]. The current study attempts to bridge the gap between folklore herbal tradition and contemporary oral hygiene by developing and assessing an herbal mouthwash enriched with the antioxidant capabilities of neem, mint, Bakul, and tulsi extracts. The research strives to explain the antioxidant activities of the herbal preparations through a thorough investigation, using a range of biochemical assays and in vitro models. The study also aims to investigate the possible implications of these formulations in the promotion of oral health through the reduction of oxidative stress-mediated damage in the oral cavity.

MATERIAL AND METHODS

Collection of Plants: Fruit of *Mimusops elengi* (Bakul), Leaves of *Azadirachta indica* (neem), *Mentha longifolia* (mint), and *Ocimum sanctum* (tulsi) were randomly collected from the campus of Ganpati Group of Institutions, Bilaspur, Yamuna Nagar, Haryana. Saccharine, PEG 40, Ethanol, and Glycerol, bought at a Yamuna Nagar store. In this analysis, only high-quality, analytical chemicals were employed.

Methods of Mouthwash Preparation:

Each component will be measured out according to its weight. The extracts were correctly blended with a modest amount of water using a mortar and pestle. The other ingredients will be added gradually while being thoroughly blended (Table 1).

Table 1: Method of mouthwash

Ingredient	Function	Formulation
<i>Mimusops elengi</i> (ME)	Active drug	250 mg
<i>Azadirachta indica</i> (AI)		250 mg
<i>Mentha longifolia</i> (ML)		250 mg
<i>Ocimum sanctum</i> (OS)		250 mg
Saccharine	Sweetener	0.1 mg
PEG 40	Surfactant	6 g
Glycerol	Co- Surfactant	6.5 ml
Alcohol	Preservative	2 ml
Purified water	Q. S	Up to 100 ml

Phytochemical Evaluation:

The crude extracts were subjected to preliminary phytochemical studies using various chemical tests for the detection of alkaloids, amino acids, carbohydrates, fixed oils, glycosides, tannins, phenols, phytosterols, protein and saponins [35]. Phytochemical analysis of all hydroalcoholic extracts carried out by the standard method [36].

In-vitro antioxidants:

Various in-vitro antioxidant tests like estimation of total phenolic compounds [37], assay of H₂O₂ scavenging activity [38, 39], Nitric oxide radical scavenging activity [40-42], (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity [43, 44], total phenolic compounds [45-49] was done according to standard procedures.

Evaluation Tests for Mouthwash:

The various evaluation tests for mouthwash are given below:

Organoleptic Properties: The organoleptic properties of the mouthwash, such as taste, colour, and smell, were checked by tasting, visual examination, and smelling [50].

pH: The pH of the mouthwash was checked by using the pH meter. The pH of the mouthwash should be between 5.5 to 7.9 [51], so that when the preparation is consumed, it does not cause any type of irritation to the oral mucosa.

Viscosity: Vertically on a suitable platform, the viscometer was installed [52].

The viscometer was filled with water up to the point indicated by the mark A.

The amount of time that it took for water to flow from point A to point B was measured and recorded.

The same process was carried out with the mouthwash as well, and the viscosity may be determined by using the formula that is provided below.

η_1 can be calculated using the formula below:

The exact same process was carried out with the mouthwash as well, and the viscosity may be determined by using the formula that is provided below.

η_1 can be calculated using the formula below.

$\eta_1 = \frac{f_1 t_1}{f_2 t_2} \times \eta_2$

f_1 = density of an unidentified fluid

t_1 = Period of unidentified fluid flow

f_2 = Normal liquid density

t_2 = Standard liquid flow time

η_2 = Normal liquid viscosity

Density: The Density of the mouthwash can be checked by the following procedure [53]:

Firstly, take a empty gravity bottle and weigh it on the weighing balance.

Now add 50ml of the mouthwash into it and weigh it.

By subtracting the weight of empty gravity bottle from the weight of the gravity bottle containing mouthwash. We will get the mass of the

mouthwash. Now by using the density formula we can get the density of the mouthwash. Density = Mass of the mouthwash/Volume of the mouthwash.

RESULTS

The various phytochemicals, such as saponins, flavonoids, terpenoids, phenols, amino acids, fixed oil, and phytosterols, were present. Other phytochemicals, such as alkaloids and glycosides, were absent. Phytochemical evaluation: The results of preliminary phytochemical screening of different plant extracts are as follows (Table 2):

Table 2 : Preliminary phytochemical screening

Chemical Constituents	Test	Colour	Extracts			
			ME	AI	ML	OS
Alkaloids	Mayers test	Cream	-	-	-	-
Saponins	Foam test	-	+	+	+	+
Glycosides	Borntrager test	Reddish pink	-	-	-	-
Flavonoids	Alkaline	Yellow	+	+	+	+
Steroids/Terpenoids	Salkowski test	Red/Yellow	+	+	+	+
Tannins/Phenols	Ferric chloride test	Blue/Black	+	+	+	+
Carbohydrates	Benedict's test	Red ppts	+	-	-	+
Proteins /Amino acids	Ninhydrin test	Violet colour	+	+	+	+
	Biuret test	Purple	+	+	+	+
Fixed oil	Spot test	-	+	+	+	+
Phytostereols	Liebermann-Burchard test	Green/Red	+	+	+	+

Antioxidant activity

Total phenol content

Extraction with hydroethanol yields a total phenol content of *Azadirachta indica*, *Mentha longifolia*, *Mimusops elengi* Linn, and *Ocimum sanctum* max were found to be 515.7 mg/ml, 508.7 mg/ml, 508.7 mg/ml, and 512.7 mg/ml Gallic acid equivalent (GAE), respectively (Figure 1).

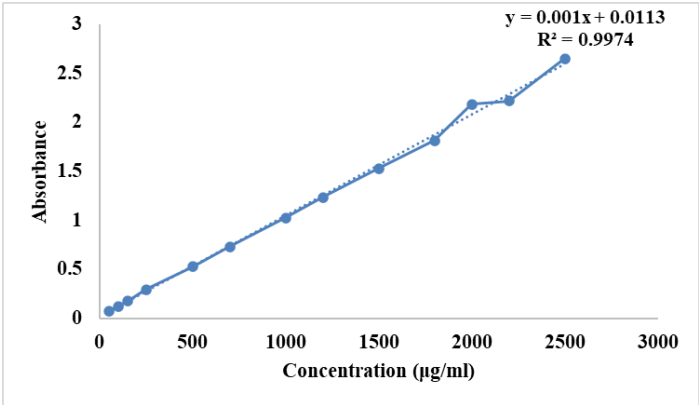


Figure 2 : Total phenol content

Hydrogen peroxide-scavenging activity:

Hydroxyl radicals are generated in cells by hydrogen peroxide. The extract's antioxidant activity is measured by its ability to quench these free radicals. A decrease in absorbance as a function of increasing concentration of the test substance is indicative of the suppression of these radicals. Scavenging tests for hydrogen peroxide, IC50 value of hydroethanolic extracts of *Azadirachta indica*, *Mentha longifolia*, *Mimusops elengi* Linn, and *Ocimum sanctum* extract were 218.56, 216.78, 221.08, and 213.55 µg/ml while that of ascorbic acid was found to be 148.84 µg/ml (Figure 2).

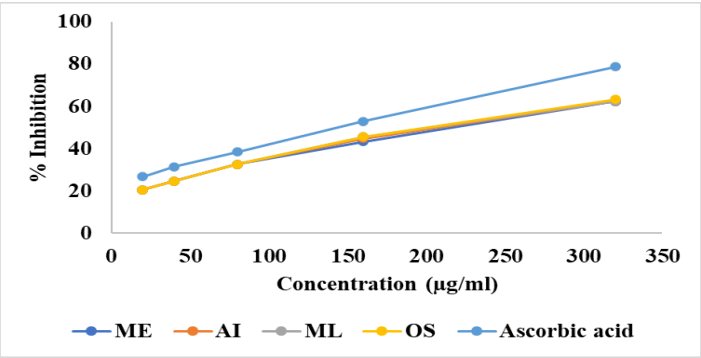


Figure 1: Hydrogen peroxide-scavenging activity

DPPH free radical scavenging activity:

Hydroethanolic extracts of *Azadirachta indica*, *Mentha longifolia*, *Mimusops elengi* Linn, and *Ocimum sanctum* were tested for their ability to neutralise the stable radical DPPH by either giving hydrogen to it or scavenging for it.

Concentrations of extract from 20 to 320 g/ml have progressively higher scavenging effects on the DPPH radical. Because of their hydrogen-donating properties, hydroethanolic extracts of *Azadirachta indica*, *Mentha longifolia*, *Mimusops elengi* Linn, and *Ocimum sanctum* decolorized DPPH. The IC50 value of hydroethanolic extracts of was determined using the DPPH free radical scavenging assay of *Azadirachta indica*, *Mentha longifolia*, *Mimusops elengi* Linn, and *Ocimum sanctum* extracts was found to be 199.87, 200.03, 209.26 and 203.7 µg/ml, while that of ascorbic acid was found to be 125.82 µg/ml (Figure 3).

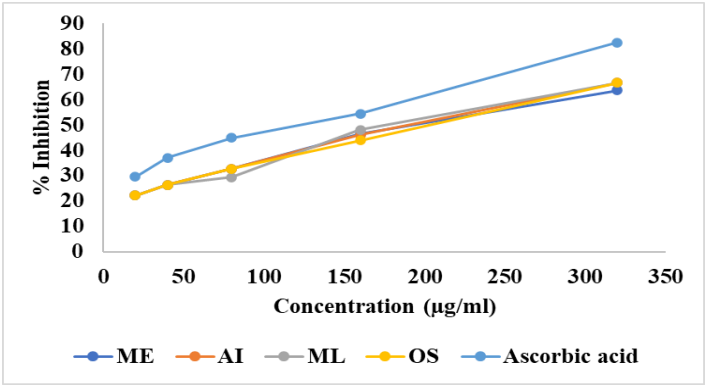


Figure 4: DPPH free radical scavenging activity

Nitric oxide radical scavenging activity

Griess reagent was used to test for nitric oxide scavenging activity. After preparing 5mM of Sodium nitroprusside in phosphate buffer saline (PBS), we combined it with 3.0 ml of extract at concentrations ranging from 20 to 320 g/ml, and then we incubated the combination at 25°C for 150 minutes [54, 55]. In NO scavenging activity, IC50 value of hydroethanolic extracts of *Azadirachta indica*, *Mentha longifolia*, *Mimusops elengi* Linn, *Ocimum sanctum* extracts was 203.9, 206.8, 203.25, and 209.94 µg/ml while that of ascorbic acid was found to be 155.9 µg/ml (Figure 4).

Physical Evaluation of Mouthwash:

Tables 3 and 4 include data on organoleptic evaluation, viscosity, and pH. Organoleptic evaluation involves the visual sense, smell, and taste (Table 3) (Table 4).

Alterations in mouthwash's colour, smell, and flavour are all part of the physical examination. If the mouthwash is to be eaten without irritating the oral mucosa, its pH value must be within the 5.5–7.9 range, which is the normal range of the oral cavity.

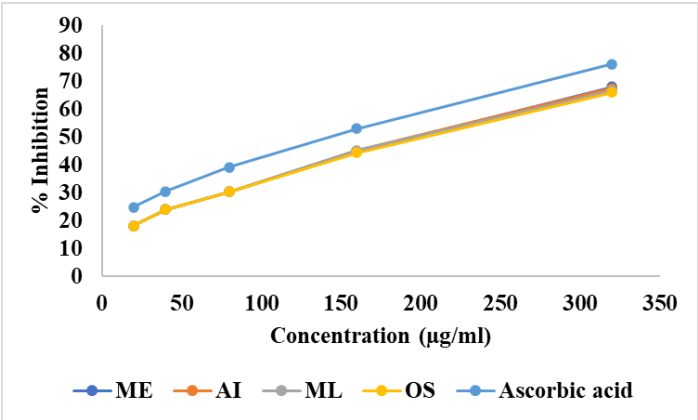


Figure 3: Nitric oxide radical scavenging activity

Table 3: Organoleptic evaluation of Mouthwash

Color	Taste	Smell
Yellow	Bitter	Menthol

Table 4: Result of Physical Evaluation

Viscosity	Density	ph
1.003	1.103	6.16

The rate at which a liquid move through a cylindrical tube can be used as a measure of its viscosity. The thickness, or viscosity, of a material. It takes more energy to move a liquid of higher viscosity than one of lower viscosity. The Oswald viscometer was used to determine the exact viscosity of the mouthwash made from a citronella leaf infusion. Measurements of the four different formulations revealed that the mouthwash preparations had different viscosities, revealing that the unit of viscosity of a solution is the dispersion medium. A liquid's specific gravity determines its viscosity; hence, a larger value indicates a higher viscosity.

CONCLUSION

In recent years, the traditional remedy has seen widespread adoption in the pharmaceutical industry. Because of their low cost (relative to conventional pharmaceuticals) and lack of negative side effects, herbal remedies are increasingly popular. Because of this need, scientists are now putting traditional assertions through rigorous scientific scrutiny. That's why it's crucial to have a pharmacognostic evaluation of herbal medicines as a criterion for standardisation. This research will aid in the future monograph creation of the crude drugs neem (*Azadirachta indica*), mint (*Mentha longifolia*), elengi (*Mimulus elengi*), and sanctum (*Ocimum sanctum*) (tulsi).

The mouthwash formulation of *Azadirachta indica* (neem), *Mentha longifolia* (mint), *Mimusops elengi* Linn, and *Ocimum sanctum* (tulsi) has the potential effect as a herbal mouthwash, according to the results of numerous evaluation tests.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Conflict of interest

No conflicts of interest to declare.

Declaration of Competing Interest

The authors assert that they do not have any known financial interests or personal relationships that could be perceived as influencing the work reported in this paper.

Funding

There is no funding agency in this article.

Data availability

Not applicable.

REFERENCES

1. Polzer, I., et al., *Edentulism as part of the general health problems of elderly adults*. International Dental Journal, 2010. **60**(3): p. 143-155.
2. Gift, H.C. and K.A. Atchison, *Oral health, health, and health-related quality of life*. Medical care, 1995: p. NS57-NS77.
3. Gift, H.C. and M. Redford, *Oral health and the quality of life*. Clinics in geriatric medicine, 1992. **8**(3): p. 673-684.
4. Jin, L., et al., *Global burden of oral diseases: emerging concepts, management and interplay with systemic health*. Oral diseases, 2016. **22**(7): p. 609-619.
5. Curciarello, R., et al., *Contribution of non-immune cells to activation and modulation of the intestinal inflammation*. Frontiers in Immunology, 2019. **10**: p. 647.
6. Ramos-Lopez, O., et al., *Epigenetic signatures underlying inflammation: An interplay of nutrition, physical activity, metabolic diseases, and environmental factors for personalized nutrition*. Inflammation Research, 2021. **70**: p. 29-49.
7. Levitt, B.B., H.C. Lai, and A.M. Manville, *Effects of non-ionizing electromagnetic fields on flora and fauna, part 2 impacts: how species interact with natural and man-made EMF*. Reviews on Environmental Health, 2022. **37**(3): p. 327-406.

8. Appanna, V.D. and V.D. Appanna, *Dysbiosis, probiotics, and prebiotics: in diseases and health*. Human Microbes-The Power Within: Health, Healing and Beyond, 2018: p. 81-122.
9. Al-Gubory, K.H., P.A. Fowler, and C. Garrel, *The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes*. The international journal of biochemistry & cell biology, 2010. **42**(10): p. 1634-1650.
10. Juan, C.A., et al., *The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies*. International Journal of Molecular Sciences, 2021. **22**(9): p. 4642.
11. Chauhan, S., et al., *Antihyperglycemic and Antioxidant Potential of Plant Extract of Litchi chinensis and Glycine max*. International Journal of Nutrition, Pharmacology, Neurological Diseases, 2021. **11**(3): p. 225-233.
12. Sroussi, H.Y., et al., *Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis*. Cancer medicine, 2017. **6**(12): p. 2918-2931.
13. Zamora-Perez, A.L., et al., *Increased micronuclei and nuclear abnormalities in buccal mucosa and oxidative damage in saliva from patients with chronic and aggressive periodontal diseases*. Journal of Periodontal Research, 2015. **50**(1): p. 28-36.
14. Vo, T.T.T., et al., *The promising role of antioxidant phytochemicals in the prevention and treatment of periodontal disease via the inhibition of oxidative stress pathways: updated insights*. Antioxidants, 2020. **9**(12): p. 1211.
15. Dhankhar, S., et al., *Novel targets for potential therapeutic use in Diabetes mellitus*. Diabetology & Metabolic Syndrome, 2023. **15**(1): p. 17.
16. Bodet, C., et al., *Potential oral health benefits of cranberry*. Critical Reviews in Food Science and Nutrition, 2008. **48**(7): p. 672-680.
17. Scardina, G.A. and P. Messina, *Good oral health and diet*. BioMed Research International, 2012. **2012**.
18. König, K.G. and J.M. Navia, *Nutritional role of sugars in oral health*. The American journal of clinical nutrition, 1995. **62**(1): p. 275S-282S.
19. Ghutke, T.D., et al., *A Comprehensive Review on the Therapeutic Properties of Medicinal Plants*. Acta Traditional Medicine. V2i01, 2023: p. 13-00.

20. Kumari, N. and R. Kumari, *A study of taxonomy and ethnopharmacological role of medicinal plants in the health care system of the tribal blocks of district Gopalganj Bihar*.
21. Dixit, U., *Traditional knowledge from and for elderly*. 2011.
22. Mittal, P., et al., *A Review on Natural Antioxidants for Their Role in the Treatment of Parkinson's Disease*. Pharmaceuticals, 2023. **16**(7): p. 908.
23. Samrat Chauhan, L.K., Navpreet Kaur, Randhir Singh, *Potential Anti-Arthritic Agents From Indian Medicinal Plants*. Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences, 2015. **4**(3): p. 10-22.
24. Lalit, K., et al., *Phyto-pharmacological review of Coccinia indica*. World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS), 2014. **3**(2): p. 1734-1745.
25. Emmanuel, S.D., et al., Potential therapeutic option used for the cure of covid-19 using locally available indigenous herbs (nigeria) containing antioxidant, vitamins, minerals; thus, this will help to tackle current status, challenges as well as futuristic perspective globally. 2020.
26. Saeed, K., et al., Application of essential oils in food industry: challenges and innovation. Journal of Essential Oil Research, 2022. **34**(2): p. 97-110.
27. Bhavaniramy, S., et al., Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain & oil science and technology, 2019. **2**(2): p. 49-55.
28. Adwas, A.A., et al., Oxidative stress and antioxidant mechanisms in human body. J. Appl. Biotechnol. Bioeng, 2019. **6**(1): p. 43-47.
29. Pisoschi, A.M., et al., Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry, 2021. **209**: p. 112891.
30. Assiry, A.A., et al., Crossover analysis of the astringent, antimicrobial, and anti-inflammatory effects of Illicium verum/star anise in the oral cavity. BioMed research international, 2021. 2021: p. 1-6.
31. Abdel-Karim, O.H., et al., Phytochemical Screening and antioxidant activity of Chlorella vulgaris. Delta Journal of Science, 2019. **41**(1): p. 79-91.
32. Dakshayani, L., et al., Holy basil: A potential herbal source for therapeutic applications. Current Trends in Biotechnology and Pharmacy, 2021. **15**(1): p. 87-100.
33. Rao, D.P. and A. Kumar, *Ocimum Tenuiflorum's Impact As An Immunostimulant In Labeo Rohita (Hamilton)*. Journal of Experimental Zoology India, 2023. **26**(2).
34. Esmeeta, A., et al., *Plant-derived bioactive compounds in colon cancer treatment: An updated review*. Biomedicine & Pharmacotherapy, 2022. **153**: p. 113384.
35. Ramamurthy, V. and M. Sathiyadevi, *Preliminary phytochemical screening of methanol extract of Indigotera trita Linn*. J. Plant. Biochem. Physiol, 2017. **5**(2).
36. Kumar, G.P. and S.N. Subrahmanyam, *Phytochemical analysis, in-vitro screening for antimicrobial and anthelmintic activity of combined hydroalcoholic seed extracts of four selected folklore indian medicinal plants*. Der Pharmacia Lettre, 2013. **5**(1): p. 168-176.
37. Sharma, G.N., et al., *Phytochemical screening and estimation of total phenolic content in Aegle marmelos seeds*. International Journal of Pharmaceutical and Clinical Research, 2011. **2**(3): p. 27-29.
38. Gupta, P., et al., *Evaluation of effect of alcoholic extract of heartwood of Pterocarpus marsupium on in vitro antioxidant, anti-glycation, sorbitol accumulation and inhibition of aldose reductase activity*. Journal of traditional and complementary medicine, 2017. **7**(3): p. 307-314.
39. Zouheira, D., et al., *In vitro antioxidant properties and inhibitory effect of extracts and fractions of Plectranthus glandulosus leaves on copper sulfate (CuSO₄)-induced oxidation in human low-density lipoprotein*. Journal of Drug Delivery and Therapeutics, 2020. **10**(4): p. 133-145.
40. Shukla, S., et al., *Antioxidant activity and total phenolic content of ethanolic extract of Caesalpinia bonducella seeds*. Food and chemical Toxicology, 2009. **47**(8): p. 1848-1851.
41. Saha, K., et al., *Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants*. Journal of Ethnopharmacology, 2004. **92**(2-3): p. 263-267.
42. Thangam, R., et al., *C-Phycocyanin from Oscillatoria tenuis exhibited an antioxidant and in vitro antiproliferative activity through induction of apoptosis and G0/G1 cell cycle arrest*. Food chemistry, 2013. **140**(1-2): p. 262-272.
43. Sun, Y., et al., Optimizing the extraction of phenolic antioxidants from kudingcha made from Ilex kudingcha CJ Tseng by using response surface methodology. Separation and purification technology, 2011. **78**(3): p. 311-320.

44. Ardestani, A. and R. Yazdanparast, *Antioxidant and free radical scavenging potential of Achillea santolina extracts*. Food chemistry, 2007. **104**(1): p. 21-29.
45. Al Harthi, S., et al., *Quantification of phenolic compounds, evaluation of physicochemical properties and antioxidant activity of four date (Phoenix dactylifera L.) varieties of Oman*. Journal of Taibah University Medical Sciences, 2015. **10**(3): p. 346-352.
46. Priya, B., et al., *In-vitro antioxidant activity and determination of total phenolic, flavonoid contents of stems of Rotula aquatica Lour.* International Journal of Pharmaceutical Sciences and Research, 2013. **4**(9): p. 3608.
47. Genwali, G.R., P.P. Acharya, and M. Rajbhandari, *Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity*. Nepal journal of science and technology, 2013. **14**(1): p. 95-102.
48. Maurya, S. and D. Singh, *Quantitative analysis of total phenolic content in Adhatoda vasica Nees extracts*. International Journal of PharmTech Research, 2010. **2**(4): p. 2403-2406.
49. Barku, V., et al., *Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of Amaranthus spinosus*. 2013.
50. Dudhe, S.B. and C.R. Doijad, *Formulation and evaluation of Herbal toothpowder*.
51. Singh, M., et al., *Formulation, Development, and Evaluation of Herbal Effervescent Mouthwash Tablet Containing Azadirachta Indica (Neem) and Curcumin for the Maintenance of Oral Hygiene*. Recent patents on drug delivery & formulation, 2020. **14**(2): p. 145-161.
52. Chaudhary, P., et al., *Preparation and Evaluation of Herbal Mouthwash Containing Hydroalcoholic Extract of Pongamia pinnata*. Asian Journal of Biological and Life Sciences, 2023. **12**(1): p. 173.
53. Anshula, D., et al., *Evaluation of the stability, pH, density and sedimentation of green tea and green tea plus ginger mouthwash: A phytochemical study*. J Oral Health Dent, 2017. **2**: p. 102.
54. Shukla, S., et al., *In vitro antioxidant activity and total phenolic content of ethanolic leaf extract of Stevia rebaudiana Bert.* Food and Chemical Toxicology, 2009. **47**(9): p. 2338-2343.
55. Marcocci, L., et al., *The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761*. Biochemical and biophysical research communications, 1994. **201**(2): p. 748-755.