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Discussion of Phytochemistry and Pharmacological Properties of Commonly used Spices as Active Ingredients in Tooth Paste Formulation

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Abstract - *Medicated formulations that have plants as their derivative are widely popular due to their safety point of view, easy availability and cost efficiency. These medicines can be procured orally, inhaled or straightforwardly applied to the skin as topicals. Therapeutic herbs are progressively critical to the strength of individuals and communities. The therapeutic effect of these plants lies in bioactive phytochemical constituents that exhibit positive physiological activities on the human body. Utilization of plant-based medications for relieving different infirmities is as old as human progression and is utilized in all societies since time immemorial. Therefore, plants having high content of bioactive compounds and antioxidants can be formulated to various products like nutraceuticals and food supplements, commercial products like herbal hair oil, polyherbal toothpaste, natural anti-acne creams, herbal face wash and into several other fully or partially organic formulation.*

In this review we discuss to formulate a polyherbal toothpaste using plant extracts of commonly used spices like Piper nigrum (Black pepper), Laurus Nobilis (Bay leaves), Syzygium aromaticum (Clove) and Amomum subulate (Black cardamom). In the current study phytochemistry of the selected plant samples has been thoroughly accessed via qualitative and quantitative analysis. Further studies involve the evaluation of antioxidant content, anti-inflammatory effects and anti-bacterial activity by various assays and tests. And lastly, validation of the final product formed on different parameters like pH, durability, spreadibility etc.

Key Words: Medicated Formulation, Therapeutics herb, Bioactive compounds, Antioxidants, Polyherbal

1. INTRODUCTION

Rapid modification in lifestyle, living and urbanization had marked the emergence of several severe health hazards across the world. Diseases related to oral fissures pose a major health issues for many countries especially with low and medium economic income. In most of the cases once the patient gets infected with oral related diseases, it becomes very difficult to get rid of it. Majority of the patients experience extreme pain and disfiguration all throughout their life. In some cases, especially oral cancer can lead to removal of certain oral parts like tongue, vocal cord and jaws. In extremely serious cases, it can even cost lives of individuals. Some factors contributing to this health risk includes consumption of drugs, alcohols, gutkas, pan masalas, tobacco and intake of high content of sugar diets.

Toothpastes are basically dentifrice, that has been used since ages and over the generations with the ultimate purpose of serving good oral health care (Mangilal and Ravikumar, 2016). These gels are utilized with a motive to protect and maintain oral hygiene. It usually has a refreshing and sweet taste that gives individuals a sense of freshness. It uses detergents, fluorides and several other chemicals as active ingredients (Banani et al., 2013).

Recent reports of World Health Organization (WHO) suggests that "around 3.6 billion people (which is nearly half the world population) is suffering from oral diseases" (Spencer et.al, 2017). "Amongst which dental caries in permanent adult teeth being extremely relevant. About 5.3 lakhs of children are suffering from milk teeth decay" (Benjian et.al, 2014). "Studies also reveal that approx 10 - 12% of the entire population have fallen prey to severe gingivitis and periodontal tooth conditions" (Bhawna, 2013). "Estimates of Ministry of Health and Family welfare reports that in India 50% of youth (especially children) experience severe tooth decay problems and 90% of Indian citizens have illness related to periodontist" (N. Kishor, 2010). Moreover, our country stands at third position in oral cancer (Jacques et.al, 2018).

The most common problems related to oral fissure includes tooth sensitivity, building of bacteria on tongue and teeth, gingivitis, noma (prevalent in malnutrient and under nourished children), periodontitis, oral cancer, cleft lip and palate (genetic disorder) dry mouth, trench mouth and calculus formation. The most prevalent etiological agent for dental caries is Streptococcus mutans. Along with this other microbe such as Staphylococcus aureus, Treponema denticola, Porphyromonas gingivivalis and Actinomycetes play a crucial role in conditions of severe periodontis (Loesche, 1996). Apart from these Candida (yeast), Prevotella intermedia, Treponema vincentii and various species of Fusabacterium cause oral thrush (in infants) and trench mouth respectively (Conrads et.al, 2011).

1.1 Justification

In concern with oral related diseases and disorders: "Prevention is always better than cure". Use of efficient and effective toothpastes that significantly cures oral problem plays a crucial preventive role. But most of the tooth dentifrice available in commercial markets contain harmful and suspicious chemicals like surfactants, substances of abrasive nature and fluoride derivatives. Prolong consumption of these contagious tooth products may lead to extremely severe health issues especially disorders related to oral cavity. And it can even prove to be lethal, if consumed in extensive quantities (Fejerskov, 2016). Research papers suggests that "consumption of 15-16 mg of tooth dendririces/kg of body weight can prove deadly for adult individuals and approximately 6mg of tooth paste consumption/kg of body weight can be lethal for small children" (Davies,et.al, 2008)

Having an insight to these severe problems, this project aims to formulate a poly herbal tooth paste that is fully organic in its nature. The safety of the product formulated is kept at utmost priority. Herbal tooth pastes are chemical free. Their ingredients are fully natural especially comprises of extracts of medicinal plants and spices that have profound medicinal uses over the generations. These crude extracts obtained from plants are highly rich in phytocompounds and essential oils that supplements aroma, flavor and characteristic properties to the finally formulated product.

2. Review of Literature

2.1. Classification of Herbal medicince

Ayurvedic herbalism (Ayurveda meaning science of life) which is gotten from Sanskrit word. This framework began from Indian meds around 500 quite a while back and was rehearsed in its neighboring nations like Sri lanka (Saini and Anu, 2016).

Chinese herbalism – Also known as Yin Yang, is believed to balance out the energies of the body that runs throughout various organs. It is a healing system, wherein the medical herbs used since ages in China are known to have powerful healing actions (Zhu et.al, 1995)

African herbalism – Based on the grounds of cultural practices and tradition believes. It was believed that healing from illness, can be achieved in combination to spiritualism, divinity and knowledge of medical herbs (Ozioma et.al, 2019)

Western herbalism - Greece and Rome were their beginning nations which were then spread to Europe, North and South America. Plants based medication assume a significant job in world wellbeing. Restorative plants are dispersed worldwide however they are exceptionally wealthy in tropical nations. Current prescription from high plants either legitimately or in a roundabout way inferred is evaluated 25% (Cassileth, 1998; Crag et al., 2001). Home grown drugs may be considered as "weakened medications". Each herb is not the same as other herb; a few herbs are protected and viable for determined use while others are definitely not. As each piece of plant will have numerous dynamic constituents and a portion of its constituents might be harmful, nonetheless, a high portion is required to cause poisonous quality since they are not strong.

Plants inferred meds are far predominant than the all-around characterized drugs. For instance, the quality and accessibility of crude materials is constantly an issue, the standards of quality are obscure and furthermore the control, for example institutionalization and smoothness of marketing is not all that much simple.

It basically involves the new age herbalism that requires scientific and experimental approach. It attempts to collaborate new age science with traditional believes and knowledge that have been long mentioned in the Vedas, where plant extracts of different sources having healing properties, were mixed in required and appropriate amounts to formulate a "syrup" that helped to relief a patient from illness (Niemeyer et.al, 2013).

Natural meds are far predominant than the engineered drugs since they are normally happening, effectively accessible without cost and have least side impacts. Dominant part of plants have restorative properties, for example most pharmaceutical medications are initially determined from plants. The logical investigation of indigenous prescriptions is called Ethno pharmacology, which is an interdisciplinary science rehearsed everywhere throughout the world (Trenerry et.al, 2010). Institutionalization home grown readiness is named as phototherapeutic operators or pytho-drug which contains dynamic constituents, or complex blend of plant materials in the crude or prepared structure. The cutting edge field of phytoscience include the utilization of restorative plants furthermore, their bioactive phytochemicals. This science is created from converging of tremendous scope of orders that have never been connected joining a few various territories of financial, organic chemistry, physiology, microbiology medications and agribusiness.

The advancement and presentation of new medications like anti-microbials, immunoenergizers and against tumor operators have prompted emotional accomplishment in charge of numerous illnesses. The medications got from plants, in any case, still from the backbone of restorative treatment in the creating nations. As indicated by the June 1983, issue of world Health, it is assessed that more than half of the total population depends chiefly on conventional cures. Regular items and the therapeutic operators got there from, are additionally an basic component in the human services arrangement of the staying of the population living for the most part in created nations, More than half of all medications in clinical use have a characteristic item root.

Common items keep on playing a significant job in tranquilize revelation projects of the pharmaceutical industry and other research associations. In excess of 600 herbal things have been perceived in different releases of the United Satisfies Pharmacopeia. The certainty that the greater part.

2.2. Phytochemical

The world is blessed with self borne medical plants. Medical plants are currently more in demand than ever before. It is so because with time scientist and layman are realizing their values and the huge abilities they posses that will definitely prove to be boon for the societies in present and in further generations. More precisely in the genres that favors' well being of mankind particularly in focus to medication and pharmacology. The reviving abilities, that these plants have is due to the presence of phytochemical constituents that is responsible for positive pharmacological activities on human body. Probably the most noteworthy bioactive phytochemicals are alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and furthermore (Prakashet.al, 2009). These compounds serve as foundation of present day physician recommended tranquilizes of which we are well aware. Phytochemicals are characteristic compounds that are naturally borne by plants in their vegetative products like vegetables, fruits, seed and other organic products (Yancui, et al, 2016). These compounds are bioactive in nature that works by interacting and supplementing with other factors to act against illnesses or all the more explicitly to secure recovery from illnesses (Abdelkarim, et al, 2014). Phytochemicals are for the most part partitioned into two gatherings. Essential constituents contain normal sugars, amino acids, proteins and chlorophyll while optional constituents contain alkaloids, flavonoids, saponins, tannins, phenolic mixes and numerous more.

2.2.1 PHENOLS

Phenols are wide spread in nature and are likely the biggest gathering of auxiliary plant metabolites. It is an important secondary metabolite that is being exudate by plants under stress, structurally they incorporate one or more hydroxyl groups in the aromatic rings. They have very high content of antioxidants which helps them to reverse the effects of free radicals on the body. These also contribute to anti-cancerous, anti-defensive and organoleptic properties in the plant. Phenols might be separated into a few classes based on the source of where they derive. Some have benzoic acid as derivatives which others have cinnamic acids (Dai et.al, 2010).

Those of pharmaceutical significance are the straightforward phenolic derivatives and compound. Straightforward phenolic mixes comprise of a solitary phenolic ring and frequently have alcoholic, aldehydic what's more, carboxylic acid gatherings (Otavio, et al, 2017). Models incorporate vanillin which is a phenolic aldehyde and salicylic corrosive that is a phenolic corrosive. Vanillin is found in the unripe organic product's of different types of Vanilla. Capsacin is found in the dried ready product of various types of Capsicum. It has been utilized inside for dyspepsia and fart. Remotely, it is oftentimes utilized as counterirritant. Consumption of diets high on phenolics like carotenoids decreases inflammation, fine lines, ageing and risks associated to various CVDs and chronic diseases (Heather, et al, 2012).

2.2.2 ALKALOIDS

"More than 12,000 alkaloids, including more than 150 families, have been identified in plants; and around 20% of the 'species of flowering plants' contain alkaloids. In plants, alkaloids generally exist as salts of organic acids like acetic, malic, lactic, citric, oxalic, tartaric, tannic and other acids" (Tristan, et al, 2013). Alkaloids are bioactive secretions produced by plants especially legumes. These are nitrogen containing bioactive medically useful compounds (Zahra et. al, 2020). The situation of the nitrogen atom in the carbon ring shifts with various alkaloids and with various plant families. The position of the nitrogen atom, determines the functional property of a particular alkaloid. Some of the well known alkaloids are morphine, dopamine, serotonin and many more. These are known to have several medical uses for instance, acts as analgesics, stimulants, antidote against other dangerous poisons etc. Apart from these, it can also serve as fungicide and pesticide (Tristan, et al, 2013).

2.2.3 FLAVONOIDS

Flavonoids additionally referred to as bioflavonoids, are plant metabolites that are derivatives of phenols ad have low molecular weight. These are polyphenol antioxidant (cancer prevention agents) found normally in plants. They play a significant role in developing aroma in spices, color and fragrance in flowering plants. These biologically active substances help in seed growth and germination. Furthermore, also helps plants to cope up against conditions of stress and make them resistant and tolerant (Samanta et.al, 2011).

They are auxiliary metabolites meaning they are natural exacerbates that have no immediate contribution with the development or advancement of plants. More basically, flavonoids are plant supplements that when devoured as foods grown from the ground are nontoxic just as possibly advantageous to the human body. Till now more than 300 different flavonoids has been detached from vegetables. Flavonoids bear the responsibilities of inculcating several properties in plants like anti inflammatory, anti cancerous, anti mutagenic etc (Panche et,al 2016). Recent studies also suggest that these compounds play crucial role in replenishing the cells that are being damages as results of photosensitivity (Agati et.al, 2010). They have several applications in fields of pharmacy, cosmetics and as nutraceuticals substitutes (Panche et,al 2016).

2.2.4 TANNINS Tannins are the perplexing natural, non nitrogenous subordinates of polyhydroxy benzoic acids which are generally circulated in the plant realm. They are available in airborne parts, for example leaves, bark, natural products furthermore, stem. These are water degrading substances with astringence property. These are high molecular load molecules measuring between 500 – 3000Da; when complexed with other constituents can even weigh upto 20000Da or even more (Malgorzata, et al, 2017). These were earlier used widely in leather farms but now plays pivotal roles in fields of medicine. These also helps in maintaining bodily homeostasis', by reactively interacting with other proteins and sugars (Sieniawska and Baj, 2017).

Some phenolic substances, for example, gallic acid and catachins usually happen to be in complex with tannins furthermore, called as pseudotannins. A large portion of the genuine tannins have the atomic load in the middle of 1000 to 5000. Tannins encourage and consolidate with proteins. The protein-tannin complex is impervious to proteolytic proteins. This property is known as astringent. So far about 8000+ various types of tannic acids have been identified using different analytical methods. Tannins that are condensed upon with time and deposition show more resistance against activities of pathogens (Malgorzata, et al, 2017).

They are utilized as recuperating specialists in aggravation, leucorrhoea, gonorrhea etc. Proanthocyanidins have anti inflammatory effects against allergens and increases the production of mucin in GI tract which in turn decreases puffiness and inflammation in the bowel (Clinton and Catherine, 2009)

2.2.5 SAPONINS

The name saponin is gotten from the Latin word 'sapo', which implies the plant that comprises of foaming specialist when weakened in watery arrangement. Saponins contain polycyclic aglycones. The sapogenin or the aglycone part is either a triterpene or steroid. The mix of sapogenin, hydrophobic or fat-dissolvable, hydrophilic or water-dissolvable sugar part improves the frothing capacity of saponins (Mohan et.al, 2016). Some poisonous saponins are known as sapotoxin. Saponins are an significant gathering of glycosides which are broadly circulated as plant constituents. The majority of the saponins are unbiased and dissolvable in water. Like different glycosides, saponins are hydrolyzed to frame a sugar and an aglycon, for the most part known as sapogenin (Faisal et.al, 2019).

Saponins present in oat flakes and in certain green leafy vegetables like spinach helps in better absorption of iron and calcium ions in the body, which aids in digestion process (Cannas, 2015). These are used in the manufacturing of steroidal hormones as well as preservatives and biological surfactant due to their foaming abilities (Dorota et.al, 2017). Dehydrosoyasaponin is a naturally occurring terpenoids in the legumes of chickpeas. It acts as a ligand that facilitates movement of ions across calcium and potassium channels, this very phenomenon helps in treatment of various disorders related to brain, respiratory tract and urino genital tract (Mohan et.al, 2016). It shows properties of hemolysis which is responsible to increase membrane permeability (Kurt et.al, 2005). This very property is also responsible to accelerate intestinal mucous layer permeability which aids in the absorption of certain nutrients and mineral ions, which are usually impermeable through the gut membrane (Savage, 2003). Apart from these it also possesses potency against inflammation and arthritis (Faisal et.al, 2019).

3. Antioxidant

The primary meaning of antioxidant was proposed by Halliwell et al. in 1989 as "any substance that, present in low focuses contrasted with oxidizable substrates (sugars, lipids, proteins or nucleic acids, altogether delays or on the other hand restrains the oxidation of the referenced substrates". Afterward, different meanings of cancer prevention agent were proposed, for example, "any substance that forestalls, delays or disposes of oxidative harm of an objective particle" or "any substance that can dispense with receptive oxygen species legitimately or in a roundabout way, acting as a controller of the cell reinforcement resistance, or hindering the creation of those species.

Reactive oxygen species (ROS) are a gathering of particles delivered by some metabolic procedures, because of the activity of oxidases in the mitochondria or other cell compartments. ROS have high reactivity since they have unpaired electrons that can connect with oxidizable substrates through redox responses. The primary ROS engaged with the organic frameworks are superoxide anion, hydroxyl radical, hydroperoxyl what's more, peroxyl radical, nitric oxide, and different species, for example, hydrogen peroxide, singlet oxygen, and hypochlorous corrosive . Be that as it may, there are other receptive atoms inferred from the response of ROS with nitric oxide (reactive nitrogen species, RNS) or thiols (reactive sulfur species, RSS).

The balance among oxidants and antioxidant agents (redox balance) is fundamental in keeping up a sound cell microenvironment. The age of oxidative pressure is brought about by a change to be determined between ROS generation and the proficiency of the cell cancer prevention agent resistance framework. Cells and tissues are persistently being presented to free radicals got from the digestion or outer components, for example, contamination, microorganisms, allergens, radiation, cigarette smoke, and pesticides . Be that as it may, ROS can play a double job, going about as useful or unsafe components. On the one hand, the expansion in ROS creation produces oxidative stress, a harming procedure that can adjust cell structures and impacts the statement of qualities identified with quickened cell maturing. By the by, ROS got from the mitochondrial respiratory chain, at low or moderate fixations, partake in physiological capacities, for example, in

the protection against contaminations and in the support of redox balance.

Cells have a few components to change and kill ROS to maintain a strategic distance from their unsafe effects. The synergistic activity of both cancer prevention agent proteins and chemicals and exogenous cancer prevention agents kill free radicals and balance cell flagging. Truth be told, various examinations recommend that cancer prevention agents apply a defensive impact against radiation and furthermore forestall the advancement of numerous ailments, for example, disease, atherosclerosis, stroke, rheumatoid joint inflammation, neuro-degeneration, and diabetes.

3.1. BLACK PEPPER

Piper nigrum is usually referred to as black gold or King of aromatics. It is a perennial climber that is been grown in and around the world to fulfill its huge demand. It is the most exclusively and abundantly consumed amongst the spice aromatics. It has its origin in tropical India, more exactly in the Western Ghats and Malabar Coast. In India, Kerala since time immemorial is considered as its largest home (Ravindran, 2000). It belongs to piperaceae family and possesses a characteristic pungent taste. In foreign countries it is widely used as a seasoning aromatic with salads and all sorts of meat recipes. Apart from dietary uses, it can also be used in perfumery industries, pharma industries as active medicine ingredient and in food industries as preservatives.

It is known to have high antioxidant content. " Extracts of Piper nigrum exhibit 93 -95 % of opposition against activity of free radical" (Su lan et al, 2007). Active constituent includes alkaloid and piperine. Piperine enhances the enzymatic activity of glands associated with digestive system thereby increasing digestion capacity and reduces the transition time in GIT. There are evidences showing that it helps in reducing lipid oxidation which beneficially influences the thiol concentration in the cells. In liver, it opposes the transformation of drugs especially derived from enzymes. "It also inhibits hepatic and intestinal aryl hydrocarbon hydrolase and UDP- glucuronyl transferase, which increases the availability of numerous therapeutics like anti histamines and phytochemicals in blood by several folds" (Srinivasan, 2007).

"Extracts of black pepper are non genotoxic and contain alkyl amides. Alkyl amides suppresses the activity of NK kappa β due to which it shows anti mutagenic and anti tumour activities upto 85% against human cancerous cells" (Liu et. al, 2010). It also increases the bioavailability of essential phytocompounds like curcumine and β carotene. "A complex mixture of piperine and curmine increases the absorption of the later by 2000%" (Hewlings et.al, 2017). "Piperine also enhances the intestinal uptake of β carotene by 147% and its absorption in the GIT by 59%. β carotene is eventually converted to Vitamin A in the body hence, helps in fulfillment of Vit- A deficiency" (Supriya et.al, 2009).

"It also contains several essential oils such as trans caryophyllene, α- pinene, limonene, β- pinene, etc. These oils show significant anti bacterial and anti fungal activity" (Miloš et al.2015). Other constituents like β- caryophyllene, β- caryophyllene oxide irreversibly influence the growth and proliferative actins of cancerous cells, thereby showing anti cancerous activity. These oils are also known to have analgesic properties (Klaudyna, et al, 2016).

3.2. BAY LEAVES

Laurus nobilis mostly used as dried leaves, possesses excellent aromatic fragrances'. It has its origin to lauraceae family and flowers. It is grown in several parts of the world like India, Indonesia, Mexico, California etc. However it is known to have its initial existence in Mediterranean region. It is widely used as spice in Indian cuisines. It has extremely pungent smell and tastes bitter. It has been used as diaphoretic since ages. "Its seeds show prominent anti ulcerative properties against gastric ulcers in rats" (Afifi et.al, 1997). Also plays crucial role in immunomodulation and regulates the expression of NF kuppa B (Mary et .al, 2007).

"Crude extracts of L. nobilis shows healing and anti inflammatory properties in rats against wounds" (Shivananda et.al, 2006). "Aqueous extracts with 80% methanol shows excellent inhibition against activity of urease with IC50 values of 48.69µg/ml, hence helps in inhibiting formation of ulcers and stones in kidney" (Mahmood et.al, 2014). Bay leaves have sesquiterpene lactones as a major constituent which shows cytotoxic activities against human cancerous cell lines (Stefanoet.al, 2006). Methanolic aqueous solutions have glucosides of megastigmane and phenolic nature which inhibits production of nitric oxide in activated macrophages (Simona et.al, 2004).

L. nobilis possesses high amounts of essential oils and the total oil content in fruits and leaves has been found to be around 99.7% and 93.7% respectively (Abu Dahad et.al, 2014). The major constituents are found to be β- ocimene, β- pinene, α pinene and 1, 8- cineole. These constituents are helpful in the treatment of SARS CoV and works wonderfully against HSV-1 replication (Monica et.al, 2008). These oils also help in inhibiting tumor proliferation (Loizzo et.al, 2007). "Methyleugenol, eugenol and pinene found in extracts have been proved to have antiepileptic action against seizures" (Sayyah et.al, 2002). 1, 8- cineole exhibits prominent antifungal activity against several species of micromycetes" (Simic et.al, 2004). This very constituent also shows anti proliferative action against breast cancer (Abu Dahad et.al, 2014).

"Daily consumption of grounded bay leaves (approximately- 3 grams) reduces levels of serum glucose, triglyceride, cholesterol and LDL cholesterol by 26%, 33%, 24% and 40% respectively". Thus helps in reducing the risks associated

with Type 2 Diabetes (Goher at.al, 2007). "It also contains parthenolides which acts effectively against migrane aches" (White, 2004). It also proves boon in maintaining oral hygiene and relieves against toothache (Banani et.al, 2013). "Apart from these, this very plant is also known to have anesthetic, antibacterial, anti viral, antirheumatic, stomachi, digestant, aperative, hypertensive, soporific, carminative, bactericidal and orexigenic activities" (Peterson, 1996).

3.3. CLOVE

Syzygium aromaticum is an evergreen tree that bears aromatic flower buds. It finds its nativity to Maluku Island (Spice Island) in Indonesia and belongs to myrtaceae familia (Oliveira et.al, 2014). They are rich in anti oxidants, vitamins, fibers, essential oils, manganese and minerals. One of its major constituent is eugenol. Eugenol is a natural antioxidant that shows anti inflammatory actions and reduces oxidative damage (Enika et.al, 2010). Eugenol rich fraction aids protection against liver disease like liver enlargement, fatty liver and liver cirrhosis (Shakir et.al, 2014). Highly concentrated amounts of clove oil is capable of inducing apoptosis in 80% of cancerous cells (Vinay et.al, 2011). It also contains oleanolic acid which induces pause in cell cycle and causes death of tumorous cell Haizhou et.al (2014). Plays crucial role in the curement of chronic pruritus (Ibrahim et.al, 2017).

Cloves exhibit anti microbial activities against bacteria, viruses and certain fungi (Nzeako et.al, 2006). Methanolic extracts exhibit bactericidal effects against periodontis causing pathogens (Christine et.al, 1996). Oils extracted out of clove and formulated into mouth rinse showed good anti plaque activities and improved the conditions of bleeding gums (Kothiwala et.al, 2014). This very property attributes it to be used in oral health care maintenance. Hence, it is an active ingredient found in mouth wash and tooth dentifrices. Reports by Haizhou et.al (2014) shows that ethyl acetate extracts of S. aromaticum possesses oleanolic acid as an important ingredient, which suppresses the growth of tumour cells and induces apoptosis against them.

Reports by Minpei at.al, (2012) shows, "ethyl alcoholic extracts of this plant show PPAR-γ ligand binding activity, which helps in suppressing the levels of sugar in body in Type 2 Diabetic patients, thereby preventing genetic diabetes". Reports by Prasad et.al, (2005) also suggests that "extract acts like insulin in hepatocytes and hepatoma cells by reducing phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) gene expression". This helps in the treatment of diabetic patients. Clove oil enhances the secretion of gastric mucous in GIT that prevents against peptic ulcers (Roberto et.al, 2011).

3.4. BLACK CARDAMOM

Amomum subulatu is a small herb that grows all throughout the year and has a long life to survive. Nativity belongs to Himalaya's in the east. Best grown in hilly areas and belongs to zingiberaceae family. They are of two types: Nepal cardamom and Chinese cardamom. There is a huge list of benefits associated with it. Oils and extracts from the spice have enormous properties.

"Some of the constituents found in oil and extracts of Badi elichi are 1, 8- cineole, alpha terpineol, limonene, terpinyl acetate and many more. Out of which 1, 8- cineole constituted about 73.27%" (Bhandariet.al, 2013). "The plant also has mono-terpene hydro-carbon amounts approximately 5 – 17 % which accounts for its stomachic, carminative and diuretic properties" (Bisht et. al, 2011).

As reported by Alam et.al, 2011, "ethanolic extracts show activities against inflammation iin rats against paw edema induced by carrageanan". "Methanolic and ethyl acetate extracts contain 1, 8- Cineole and caryophyllene which is responsible to promote analgesic effect" (Shukla et.al, 2010). "Acetone and methanolic extracts of badi elichi have high phenolic content especially protocatecheuic acid, which exhibits anti diabetic activities as it helps in revival of beta langerhan cells of pancreas (Vavaiya et.al, 2010).

"Oils and extracts of plants show significant anti bacterial activity against gram positi and gram negative bacteria like S. aureus, E. coli and P. aeruginosa, due to the presence of phytocompounds like tannins, alkaloids and flavonoids" (Umesh et.al, 2010). They are also used as preventive aid against eyelid inflammation and tuberculosis as they show cardio adaptivity properties (Verma et.al, 2010). It also detoxifies the liver cells and reverses the damage caused by alcohol consumption as suggested by Parmar et.al, 2009.

It also suppresses the peroxidation of lipids in liver due to high contents of poly phenols (Uzma et.al, 2018).

As per Iqbal et.al, 2011, It can be used as a curative agent against respiratory disorders as it loosens the blood vessel, arteries and the capillaries of the lungs and the respiratory tracks by dissolving the mucous layer or the blockages. Thus, helps to improve circulation of air in lungs and also prevents blood clots (Verma et al, 2012). Moreover, also acts as expectorant and helps to expel mucous out of the body and prevents from severe cough and cold. It also helps in digestion by accelerating the effects of various digestive glands and their secretions. This not only reduces the time taken in digestion but also prevents the formation of gastric ulcers in stomach linings (Anandaraj et.al, 2011). "Cardamom exhibits gut excitatory and inhibitory effects mediated through cholinergic and Ca++ antagonist mechanisms respectively and lowers BP via combination of both pathways. The diuretic and sedative effects may offer added value in its use in hypertension and epilepsy" (Gilani

et.al, 2008). Apart from these, it has potential to remove germs and bad breath causing microbes from oral cavities (Chowdhury et.al, 2013).

4. Materials & Methods

4.1 COLLECTION OF DIETARY SPICES

Four of the commonly used dietary spices in Asian countries namely Piper nigrum, Laurus nobilis, Syzygium aromaticum and Amomum subulatum were organically grown in the hilly regions of West Bengal like Darjeeling and Kalimpong. The edible parts of these plants were harvested and processed in regard to traditional processes.

4.2 PREPARATION OF PLANT EXTRACT

The collected aromatic samples were finely grounded to powder using mortal and pistil. Once the aromatics were properly crushed, the extracts were obtained using the decoction method. For this five grams of each of the powdered samples were weighed and dissolved in 30 ml of distilled water, then kept for stirring for 45 minutes at 50°C. Allow the mixture to cool down to room temperature and centrifuge for 10 minutes at 4000rpm. The so obtained supernatants were collected in different well labeled falcon tubes which were stored at 4°C for further studies.

4.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical screening of extract of Piper nigrum, Laurus nobilis, Syzygium aromaticum and Amomum subulatum were done to detect the presence of phenolics, tannins, flavonoids, saponins, carbohydrates, proteins, coumarins, phytosterol, emodins, anthocyanins, terpenoids, alkaloids, phlobatannins, anthocyanosides, anthroquinone, steroid, oils and fats. All the tests were performed in accordance to the protocols. Qualitative analysis was further followed by quantification of certain phytocompounds.

4.4 QUANTIFICATION OF PHYTOCOMPOUNDS

4.4.1ESTIMATION OF REDUCING SUGARS (DNS METHOD)

To estimate the overall sugar content in the samples, 0µl, 10µl, 100µl, 500µl, 1000µl and 1500µl of the working standard [dextrose solution (10mg/ml)] was pipetted out into a series of test tubes with varying concentration and distilled water was used to make up the volume to 3ml. Then, 100µl of aqueous spice extracts were taken in clean test tubes and the volume was made to 3ml using distilled water. To this 2ml of DNS reagent was added in each test tubes and the test tubes were incubated in water bath for 10 minutes at 60°C. The test tubes were allowed cool to room temperature and absorbance was taken at 540nm. In this dextrose solution (10mg/ml) was used as standard (Miller and Lorenz, 1959).

4.4.2 ESTIMATION OF PROTEIN CONCENTRATION (FOLIN & LOWRY METHOD)

In order to quantify the total protein content, 0µl, 30µl, 60µl, 120µl and 240µl of the working standard [BSA (1mg/ml)] was pipetted out into a series of test tubes with varying concentration and distilled water was used to make up the volume to 1ml. Then, 50µl of aqueous plant extracts were transferred in clean test tubes and the volume was made to 1ml using distilled water. Them, 4.5 ml of Reagent 1 (combination of 48 ml of 2% Na_2CO_3 in 0.1M NaOH, 1ml of 1% sodium potassium tartarate in distilled water and 1 ml of 0.5% anhydrous cupper sulphate in distilled water) was added and incubated for 10 minutes at RT. Followed by addition of 500µl of Reagent 2 (1 part of Folin Phenol [2N]: 1 part of distilled water) and incubated it in dark for 30 minutes. Here 2ml of distilled water served as blank. A solution of BSA (1mg/ml) served as reference. After incubation immediately took the absorbance at 660nm and a standard graph was plotted to estimate the unknown concentration (Oliver H et.al, 1951).

4.4.3 TOTAL PHENOLIC CONTENT (FOLIN - CIOCALTEAU METHOD)

Total phenolic content of the aqueous aromatic extracts was determined by Folin Ciocalteau method. For this 0µl, 2µl, 20µl, 40µl, 60µl and 80µl of the working standard [gallic acid (1mg/ml)] was pipetted out into a series of test tubes with varying concentration and distilled water was used to make up the volume to 0.5ml. Then, 100µl of aqueous extracts were taken in clean and dry test tubes and was diluted by adding 400µl of distilled water to make the volume to 0.5ml, 2.5 ml of 0.2N F.C Reagent was added and incubated in dark for 5 minutes. Following 2ml of 7.5% sodium carbonate solution was added and incubated for 1 hour in dark at room temperature. The absorbance of each samples were measured at 765nm. Plot the graph with protein concentration on x- axis and absorbance on y- axis and prepare a standard graph to calculate the amount of phenolic compound present in unknown sample. Gallic acid (1mg/ml) served as standard. The result were expressed as gallic acid equivalents (mg/ml) (Siddiqui et.al, 2017).

4.4.4 TOTAL FLAVONOID CONTENT

Total flavonoid content of the aqueous spice extracts was determined by Aluminium chloride spectrophotometric method. In this 0µl, 200µl, 400µl, 600µl, 800µl, 1000µl and 1200µl of the working standard [quercetin (1mg/ml)] was pipetted out into a series of test tubes with varying concentration and distilled water was used to make up the volume to 7 ml. Then, 1ml of the samples were taken in well labeled test tubes and the volume was made to 7ml using distilled water. It was mixed with 300µl of 5 % sodium nitrate solution (2.5g in 50ml), incubated it for 5 min at room temperature. To this added 300µl of 10% Aluminum chloride (0.5g in 5ml), incubated the mixture for 6 more

minutes. Finally 2ml of 1 M NaOH solution was added. Quercetin (1mg/ml) was taken as reference. The total flavonoid content was determined from a calibration curve and the results were expressed as mg quercetin in equivalent per gram dry weight (Baba SA and Malik, 2015).

4.4.5 TOTAL TANNIN CONTENT (VAN BURDEN AND ROBINSON METHOD)

Total tannin content of the aqueous crude extracts was determined by Van Burden and Robinson method. For this 0µl, 100µl, 200µl, 300µl and 400µl of the working standard [tannic acid (1mg/ml)] was pipetted out into a series of test tubes with varying concentration and distilled water was used to make up the volume to 5ml .In this 250µl of aqueous extracts were taken in clean and dry test tubes and the volume was made to 5ml using distilled water, followed by addition of 2ml of reagent (combination of 0.1M ferric chloride, 0.008M potassium ferrocyanide and 0.1N HCl in 100ml distilled water) and absorbance was taken exactly after 10 minutes at 605nm. A test tube with 5ml of distilled water served as blank and tannic acid (1mg/ml) was used as standard. The results were expressed as mg tannic acid in equivalent per gram dry weight (Aguoru et.al, 2014).

4.5 ASSAYS FOR EVALUATION OF ANTIOXIDANTS

4.5.1 DPPH RADICAL SCAVENGING ASSAY

The stock was prepared by dissolving 24mg of DPPH in 100 ml of distilled water. 2ml of the stock solution was transferred to clean and well labeled test tubes. Now added 100µl of aqueous extract to it and gave it a vigorous shake. Allow it to be incubated for 15 min in dark. The decrease in absorbance was measured spectrophotometrically at 517 nm. In this 3 ml of stock solution served as reference (Quy Diem et.al, 2014).

% inhibition = [(Ao – At)/ Ao] × 100

Where, Ao = Absorbance of reference and At = Absorbance of the plant extract.

4.5.2 HYDROGEN PEROXIDE SCAVENGING CAPACITY

In this assay, 1000µl of centrifuged aromatic crude extract and 600 µl of 40 mM hydrogen peroxide solution (pH 7.4) were taken in well labeled sugar tubes. The above reaction mixture was allowed to interact for about 15 minutes and the absorbance was measured using spectrophotometer at 230nm. Here, phosphate served as blank and the reagent (40mM hydrogen peroxide solution in phosphate buffer) served as control. Citric acid (5mg/ml) served as standard (Ahmed et.al, 2015).

% Scavenging Activity = [(Ao – At)/ Ao] × 100

Where, Ao = Absorbance of reference and At = Absorbance of the plant extract.

4.5.3 FERRIC REDUCING ANTIOXIDANT POWER ASSAY (FRAP)

For this assay 0.5 ml of centrifuged crude extracts (2X dilution), 1 ml of 1 % potassium ferricyanide solution and 1ml of 0.2M sodium phosphate buffer (pH 6.6) were mixed together and allowed to incubate for 30 minutes at 50°C. The reaction was terminated by addition of 1ml of 10 % TCA solution and was centrifuged at 3000rpm for 10 minutes. The so obtained supernatant was diluted by adding 1ml of distilled water and 0.5 ml of

0.1 % ferric chloride solution. The absorbance was immediately measured at 700nm. The control consisted of all contents except plant sample, while 0.2 M sodium phosphate buffer (pH 6.6) served as blank (Soheila et.al, 2008).

% reducing power = [(Ao – At)/ Ao] × 100

Where, Ao = Absorbance of reference and At = Absorbance of the plant extract.

4.5.4 HYDROXYL RADICAL SCAVENGING ACTIVITY

In this assay the reaction mixture constituted of 0.5 ml of plant crude extracts, 0.2 ml of EDTA, 0.2 ml of ferric chloride, 0.1 ml of hydrogen peroxide and 0.1 ml of ascorbic acid. The above reaction mixture was incubated for one hour at 38°C. Then 1ml of 1% TBA and 2.8% of TCA was added to the same reaction mixture and was again allowed to be incubated at 100°C in boiling water bath for 25 minutes. Once the temperature of reaction mixture cooled to room temperature, the optical density was measured at 532nm. In this potassium phosphate buffer (20mM and pH 7.4) was used as blank and the control contained everything except plant sample (Kunchandy et. al, 1990).

% Scavenging Activity = [(Ao – At)/ Ao] × 100

Where, Ao = Absorbance of reference and At = Absorbance of the plant extract.

4.6 ASSAY FOR EVALUATION OF ANTI-INFLAMMATORY EFFECT

Anti-inflammatory assay by inhibition of albumin denaturation method. In this assay, 2.5ml of aqueous plant extracts (5X dilution) were taken, to which 1ml of 1% BSA solution was added and incubated at 37°C for 20 minutes. Then heated the samples at 90°C for 20 minutes. Once the samples were cooled the turbidity was measured at 660nm. 2 ml of 1% BSA solution served as control and Tris buffer (pH 7.4) served as blank. Aspirin (75mg/ml) served as standard drug (Romana et. al, 2018).

% inhibition = [(Ao – At)/ Ao] × 100

Where, Ao = Absorbance of reference and at = Absorbance of the plant extract.

5. EVALUATION OF ANTI BACTERIAL ACTIVITY

5.1 ISOLATION OF MICROBES FROM INDUSTRIAL WASTE WATER

In order to isolate microbes, to medium named as Mannitol Salt Agar (MSA) and Eosin Methylene Blue Agar (EMB) were used. Once all the constituents of the media were added to the conical flask including distilled water. The medium was autoclaved at 121°C for 20 minutes. The media was then poured to clean petri plates and allowed to cool. Once the media got solidified, 10µl of industrial waste water was properly spread with the help of a triangular spreader. The plates were sealed with parafilm and incubated at 37°C for 24 hours. The colonies so obtained were further streaked in media plates to obtain pure cultures.

5. 2 REVIVAL OF BACTERIAL CULTURES

Bacterial cultures available in Department of Life sciences, Rapture Biotech, Noida were revived over Nutrient Agar medium. Stored cultures were streaked over solid agar medium and incubated for 370C overnight. Pure bacterial colonies were observed next day and stored at 40C.

5. 3 ANTI - BACTERIAL ACTIVITY VIA WELL DIFFUSION METHOD

Nutrient broths were inoculated with bacterial strains and incubated at 37oC overnight. Wells were made on solid nutrient agar medium plates using a syringe. These wells were filled with extracts of plant sample. These broths were then inoculated over solid agar medium by spread plate method and were incubated at 370C overnight.

6. DISCUSSION AND CONCLUSION

Phytocompounds like phenolics, tannins, flavonoids, saponins, and coumarin glycosides were found to be present in the extracts of all the aromatic plants samples in varying concentrations. Apart from these carbohydrates, proteins, fats and oils were also present in recognizable amounts. Glycosides and terpenoids were found to be present in Laurus nobilis, Piper nigrum and Syzygium aromaticum in decreasing concentrations, whereas, phytosterol and emodin were found to be present only in Syzygium aromaticum. Results of carbohydrate estimation (DNS Method), protein estimation (Folin Lowry Method), phenolic estimation (Folin – Ciocalteau Method) and tannin estimation (Van Burden and Robinson Method) showed similar results, with higher concentrations found in Syzygium aromaticum, Laurus nobilis, Piper nigrum and Amomum subulatum respectively in decreasing concentrations. Results of flavonoid estimation (Aluminium Chloride Method) shows higher concentration of flavons to be present in Laurus nobilis, Syzygium aromaticum, Piper nigrum and Amomum subulatum in decreasing concentrations. In order to access the overall antioxidant profile four assays were carried out namely, DPPH radical scavenging activity (DPPH), Ferric Reducing Antioxidant Power (FRAP), Hydrogen peroxide radical scavenging activity and Hydroxyl radical scavenging activity. On the basis the results obtained we can surely be confident that these dietary samples are highly rich in anti-oxidants and phytocompounds. They show good result against ROS like DPPH, Hydroxyl radical etc. They also show excellent anti-inflammatory and anti-bacterial properties. Hence, we can surely use these dietary supplements as active ingredients in tooth paste formulation.

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