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Antimicrobial And Antioxidant Properties of Oregano, Coffee and Black Cumin Formulation

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ABSTRACT

Background: Fresh oregano has a great antibacterial agent. Coffee, also known as coffee bean consists of the dried ripe seeds of Coffea arabica Linn is a rich source of dietary antioxidants and has great anti-inflammatory properties. Nigella sativa(Black Cumin) seeds are useful for therapeutic purposes and could be developed as anticancer agent as well as for the treatment of chronic inflammatory pathologies

Aim: To assess the antimicrobial and antioxidant activity of oregano, coffee and black cumin herbal formulation.

Materials And Methods: 1g of oregano, coffee and black cumin mixed with 100 mL of distilled water and boiled for 15 minutes, filtered and again concentrated till 10mL. The antioxidant activity of this herbal formulation is assessed by mixing it with DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) assay. The anti-microbial activity is assessed by administration of this herbal plant extracts into media containing Candida albicans, Streptococcus mutans, Enterococcus faecalis, and Staphylococcus aureus.

Result: The spectrophotometer readings of antioxidant assay shows that 50% of oregano, coffee and black cumin extracts have more absorption percentage of about 90.6%.In antimicrobial activity, out of four bacteria, the maximum zone of inhibition is seen in Enterococcus Faecalis.

Conclusion: This study shows the formulation of oregano, coffee and black cumin produces good antioxidants when compared with other antioxidants. There are also good antimicrobial properties in these extracts. Hence oregano, coffee and black cumin formulations can be used as safe herbal alternatives instead of chemicals in commercially available antioxidants and antimicrobials for oral lesions.

Keywords: *Oregano, coffee, black cumin, Antioxidant, Antimicrobial*

INTRODUCTION

Ancient medicine utilized plant extracts, widely for their medicinal and therapeutic benefits. The extracts obtained from leaves, bark, root with medicinal purposes are useful and effective in treatment of various diseases(1)(2).Plant extracts proved to be effective because they interaction with special chemical receptors within the body. natural plant extracts are known for their antimicrobial, anti-inflammatory, anti-fungal and anti-cancer properties. They are also used in the purification of blood and reduce irritation of the tissues.They are also available as bark, syrups, pills, dried leaves, ointment, oil forms. These plant extracts can be used for long term treatment with lesser side effects(3).The following are such plants with wide therapeutic benefits.

Oregano, coffee and black cumin are known for their antioxidant, anti-inflammatory and antimicrobial properties. The plant Oregano vulgare, better known as Oregano belongs to the family Lamiaceae(4).Fresh oregano has a great antibacterial agent. It has phytonutrients such as thymol and carvacrol, which fight against infections such as staph. It's loaded with antioxidants that help prevent cell damage, and it's an excellent source of fiber, vitamin K, manganese, iron, vitamin E, tryptophan and calcium (5) Coffee, also known as coffee bean consists of the dried ripe seeds of Coffea arabica Linn(6).Coffee is a rich source of dietary antioxidants and is a major contributor to dietary antioxidant intake(7).Nigella sativa(Black Cumin) is an annual flowering plant with wide therapeutic effects .This plant is known by numerous names,for example black cumin(English),black caraway seeds (USA), shoniaz (Persian) and kalajira(Bengali)(8)(9).Nigella sativa seeds have significant effects against many ailments such as skin diseases, jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, diabetes, hypertension, rheumatism, intrinsic hemorrhage, paralysis, amenorrhea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema.(8) Black Cumin have the potential to be developed into dietary supplements as food preservative and for the improvement of human nutrition and health. In addition, the seeds could be useful for therapeutic purposes and could be developed as anticancer agent and as foodborne preservative as well as for the treatment of chronic inflammatory pathologies associated with overproduction of nitric oxide(10).Black cumin seeds have saponin and alpha hederine and in trace amount has carvone, limonene and citronellol, as well as prove relatively good amounts of different minerals such as a Fe,Ca,K,Zn,P,Cu.(8)

As herbal medicines are considered as safe for the treatment of various diseases with lesser adverse effects, economical, effective, relatively less toxic, extensive research is carried out in search for potent agents of plant origin for treating oral mucosal diseases.The present study was conducted to evaluate the antioxidant and antimicrobial effect of oregano,black cumin and coffee extract.Our team has extensive knowledge and research experience that has translate into high quality publications (11–26)

MATERIALS AND METHODS

Materials used

The materials used in this study includes extracted compounds containing 1g of oregano,1g of coffee and 1g of black cumin, these materials were acquired from authentic biomaterial sellers. The extracts were subjected to anti-microbial testing using Antimicrobial activity by agar well diffusion technique, and antioxidant testing using DPPH(2,diphenyl 1-picryl-hydrazyl-hydrate)assay[Figure 1].

Antioxidant testing

The preparation is done by taking a beaker of 15 ml of distilled water and 1 gm of oregano, coffee and black cumin extract and mixed together, these extracts were then heated thoroughly until the solution is reduced to about 1mL of concentration, in order to reduce the water and increase the concentration of extraction. Then the solution is cooled down, this is followed by addition of 2.50 grams of Carbopol, 0.50 gm of HPMC, and 0.95gm of NaCl, and 0.50gm of CMC, all the contents are stirred thoroughly in between the addition of the other. Determination of antioxidants was done. The solution containing oregano, coffee and black cumin is

subjected to 5 different concentrations of 10 µL, 20 µL, 30 µL, 40 µL, 50 µL in solution containing 1mL of DPPH, the solution is maintained at a room temperature for 10 mins, this is followed by boiling of the contents at 55 degree Celsius for 10 -15 mins , this solution is then subjected to spectrophotometry and analyzed for inhibition levels.

Antimicrobial Activity

Determination of antimicrobial activity is done by Agar well diffusion method was used to determine the antimicrobial activity of oregano,coffee and black cumin extracts in vitro. Agar was used to culture different micro-organisms examined in this study. Colonies of microorganisms were transferred to the agar plates using a swab, and their turbidity was visually adjusted with the broth and a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. The overall procedure of inoculum preparation and inoculation of culture media remained the same for all four bacteria. Each bacterium was inoculated on four agar plates for four respective concentrations of oregano,coffee and black cumin extracts. A hollow tube of 5 mm diameter was heated and pressed above the inoculated agar plates. It was removed immediately by making a well in the plate; likewise, four wells on each plate were made, one each for each bacteria such as Candida albicans, Streptococcus mutans, Enterococcus faecalis, and Staphylococcus aureus. Incubation was done for 24 hours. After the incubation period, plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to the nearest whole millimeter.

RESULT

The results of this study have shown a better inhibition percentage in antioxidant activity. The antioxidant activity testing was done in various levels of oregano, coffee and black cumin extracts as 10 µL, 20 µL, 30 µL, 40 µL and 50 µL in a test tube containing 1ml of DPPH. The spectrophotometer showed readings with antioxidant activity assay of oregano, coffee and black cumin extracts. The spectrophotometry readings of antioxidant assay shows that 50% of oregano, coffee and black cumin extracts have more absorption percentage of about 90.6%[Figure 2].In antimicrobial activity, out of four bacteria, the maximum zone of inhibition is seen in E. faecalis. [Figure 3].

DISCUSSION

An antioxidant is a substance that delays or inhibits peroxidative reactions. Antioxidants can affect different stages of an oxidative sequence. An antioxidant can be specific or have multiple sites of action.A number of specific reactions can be used to monitor the different molecular mechanisms of antioxidant.As peroxidative processes are involved in many degenerative physiopathologic events (aging,cancer,diabetes,atherosclerosis etc.,)a good pro/antioxidant balance is very important for health(4).The antimicrobial properties of herbal plants and their extracts have been recognized since ancient times, back to early 1900s.Now,the development of resistance via a pathogen to several of the usually used antibiotics provide a drive for additional attempts to find new antimicrobial agents to eradicate the infection and defeat the problems of resistance and side effects of the antimicrobial drugs that are used currently.

Polyphenols have assumed great importance, and in review Aruoma et al., proposed the use of antioxidant vegetable extracts both as food preservative and as prophylactic agents for some human diseases(27).Polyphenols are well known natural products known to possess several notable biological properties. In a previous work, the presence of five major phenolic compounds with antioxidant properties in hexane and methanol extracts of Oregano was reported(28).The compounds identified were protocatechinic acid and phenyl glucoside,caffeic acid,rosmarinic acid and a phenolic derivative of rosmarinic acid. The Oregano extracts had a very high polyphenol content and strong but differing antioxidant properties(5).

The major constituents of coffee bean are an alkaloid caffeine, polyphenolic compounds like

tannins and a phenolic acid namely chlorogenic acid(29)(30).Sangita et al., concluded that coffee possessed marked in vitro anti-inflammatory effect against the denaturation of protein. The effect was possibly due to the polyphenols contents of coffee.. Roasted coffee is a complex mixture of over 1000 bioactive compounds(31),some with potentially therapeutic antioxidant, anti-inflammatory, antifibrotic, or anticancer effects that provide biological plausibility for recent epidemiological associations. Medicinally, caffeine is used as a CNS stimulant, usually combined with another therapeutic agent and in analgesic preparations(32,33).Researches has explored the associations between coffee as an exposure and a range of outcomes including all cause mortality, cancer, and diseases of the cardiovascular, metabolic, neurological, musculoskeletal, gastrointestinal, and liver systems, as well as outcomes associated with pregnancy(34).Previous studies have consistently found that long-term coffee consumption is associated with a lower risk of Parkinson's disease(35).Coffee consumption was consistently associated with a lower risk of mortality from all causes of cardiovascular disease, coronary heart disease, and stroke in a non-linear relation (36).A meta-analysis showed a lower incidence of cancer for high versus low coffee consumption(37).High versus low coffee consumption was associated with a lower risk of prostate cancer(38), endometrial cancer(39), melanoma(39,40),oral cancer(38), leukaemia(37), non-melanoma skin cancer(41),and liver cancer(42) there were also significant linear dose-response relations indicating benefit(35)

Nigella sativa extract showed antibacterial effects with streptomycin and gentamicin. These findings suggested that preparations from the plant, if given with these antibacterial drugs, would enhance their efficacy(8).Nigella sativa L. (black cumin; family, Ranunculaceae) has shown that seed extracts inhibit the growth of Escherichia coli , Bacillus subtilis and Streptococcus faecalis. Methanolic extracts of Nigella sativa seeds have been reported to prevent adhesion of viable cells of Streptococcus mutans to smooth surfaces; therefore, it was suggested that this plant can be of value in preventing dental plaques and caries(43). Black cumin studies in recent years for the treatment of microbial disease has been used without any reported side effects

From the previous studies, the most effective antioxidant activity after 10 days of storage exhibited water and ethanol extracts of oregano. Compared with control it showed better the growth of Enterobacteriaceae and enterococci during storage were inhibited by ethanol extracts of oregano(44).Coffee beverages, additionally to caffeine, contains a variety of antioxidant and anti-mutagen agents including phenolic derivatives (such as chlorogenic acid and polyphenol caffeic acid) and diterpenes (such as cafestol and kahweol), that could act as carcinogenic detoxifying agents on oral and pharyngeal mucosa (29).Coffee consumption appears to have a protective benefit in oral cancer(45).Tavani et al found that different coffee beverages (caffeinated, decaffeinated and coffee) have different effects on oral cancer risk(46).Black cumin oil had a high content of polyunsaturated fatty acids and in spite of its high degree of unsaturation, the presence of phenolic compounds in Black cumin oil led to an increase in its relative oxidative stability(43).

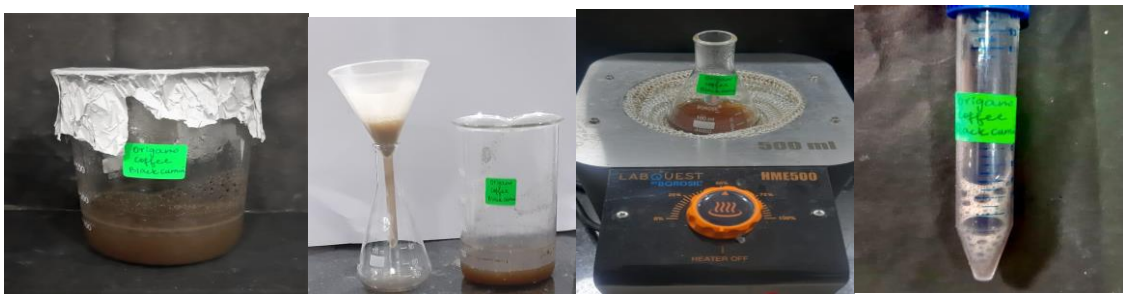

FIG 1: Processing of Oregano, Coffee and Black cumin formulation

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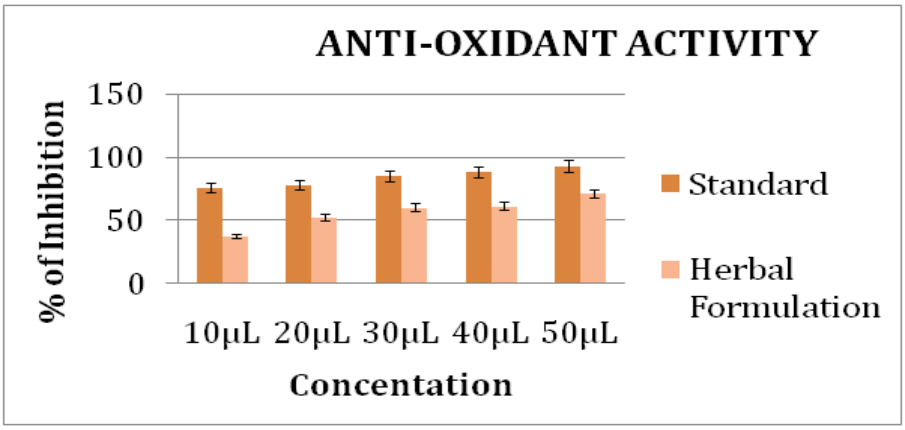

FIG 2: Shows percentage of inhibition of antioxidant assay

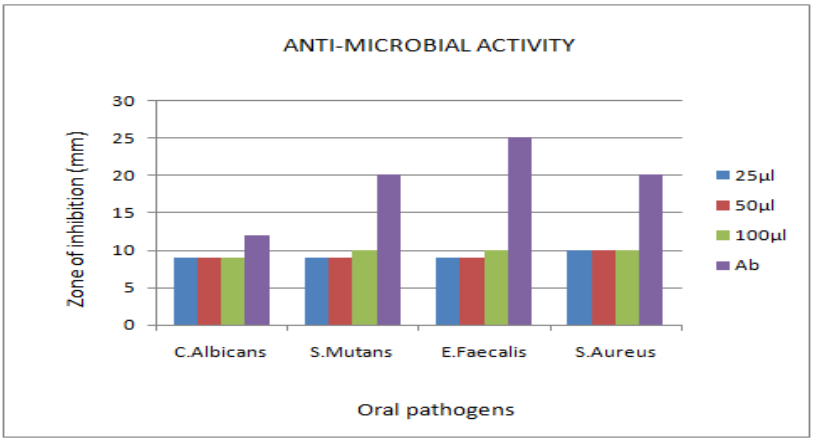

FIG 3: Shows zone of inhibition of antimicrobial assay

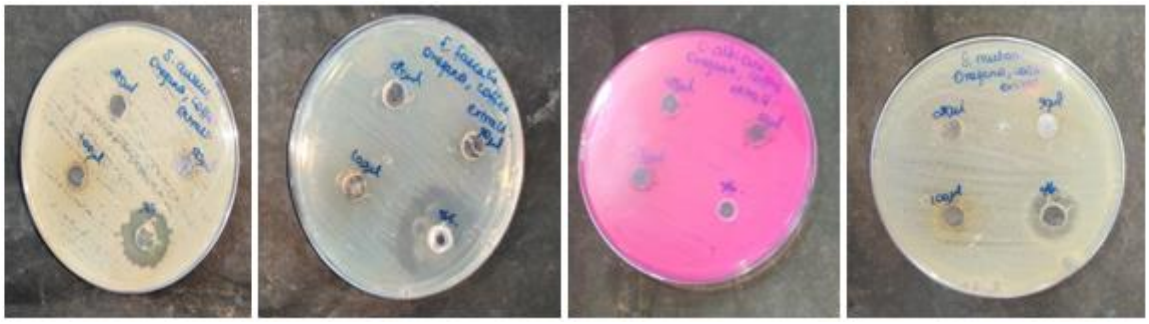

FIG 4: Antimicrobial activity assay shows zone of inhibition

CONCLUSION

The present study showed significant antimicrobial properties.This combination of extract also have a better antioxidant effect hence this study concludes that combination of Oregano,coffee and black cumin formulation has better antimicrobial and antioxidant and in future this can also be used as in-situ gel in management of oral potentially malignant disorders such oral submucous fibrosis, lichen planus and leukoplakia in the form of in-situ gel.

Clinical Significance

Oral Potentially Malignant Disorders are at increased risk for malignant transformation,

therefore early diagnosis and management of OPMDs is necessary. Many drugs seems to be effective in the management of OPMD but there are other disadvantages such as side effects of the drugs and high cost. Herbal medicine/formulation seem to have low incidence of serious adverse effects and are effective and cost efficient. Hence, the herbal formulation of oregano, coffee and black cumin as an in-situ gel seems to be promising in the treatment of Oral Potentially Malignant Disorders.

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