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Preparation of Oregano, Coffee and Black Cumin Aqueous Formulation and Its Anti Inflammatory Activity

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***Abstract*---**Background: Origanum vulgare (referred to as Spanish thyme and wild marjoram), is a member of the plant family Lamiaceae. Oregano contains potent components which contributes towards the cardiovascular and nervous systems, relieves symptoms of inflammation, and modulates blood sugar and lipids. Coffee which contains polyphenols attributes to a number of pharmacological activities that include antioxidant, antiinflammatory, immunomodulatory, anti-microbial, anti-cancer, cardioprotective and neuroprotective effects. Nigella sativa, black caraway is also called kalonji or nigella, and more common in the Far East, Mideast, Bangladesh, India and Africa. Nigella sativa contains active ingredients, in particular, thymoquinone, the main active constituent known for anti-inflammatory effect. Aim: To evaluate the antiinflammatory effect of oregano, coffee and black cumin formulation. Materials and methods: This study includes the usage of about 1g of oregano, coffee and black cumin extracts mixed with 100 mL of distilled water and boiled for 15 minutes, filtered and again concentrated till 10mL.The antiinflammatory activity of this formulation is assessed by using Bovine Serum Albumin assay. Results: The anti-inflammatory activity of oregano, coffee and black cumin aqueous formulation shows about 65% of inhibition when

compared with standard. The anti-inflammatory assay has revealed that 75% of inhibition percentage has seen in 40µl &50µl concentrations of Oregano, coffee and black cumin when compared to other levels. Conclusion: This study shows the combination of oregano, coffee and black cumin formulation produces good antiinflammatory effects. Hence oregano, coffee and black cumin can be used as safe alternatives instead of commercially available chemicals for oral lesions.

***Keywords*---**oregano, coffee, black cumin, antiinflammatory.

Introduction

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents.The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers(Chandra et al., 2012; Tripathi, 2008).Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles.The extracts obtained from the leaves, bark, root proved to be effective because they interact with special chemical receptors within the body(Mahboubi et al., 2017). They are useful and effective in various diseases. Some of the natural plant extracts are known for their anti-inflammatory, antimicrobial and antioxidant properties and are useful in treating oral mucosal lesions (Rajeshkumar et al., 2020). The various biopolymers such as Oregano,coffee and black cumin are known for their anti-inflammatory properties.

Oregano is a pervasive aromatic plant of the Lamiaceae family typical of Mediterranean flora that has been commonly used for medical purposes. Fresh oregano has a great antibacterial agent. It has phytonutrients (thymol and carvacrol), which fight 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 (Cervato et al., 2000). Coffee, also known as coffee bean consists of the dried ripe seeds of Coffea arabica Linn. (Rubiaceae). Coffee beverage is the widely consumed beverage worldwide. Coffee plant is an evergreen shrub or small tree indigenous to Arabia, grown and cultivated throughout India. Traditionally, it has been used for several important medicinal purposes in different parts of the world(Bisht & Sisodia, 2010)Among 70 species of coffee,only three are cultivated.75% of the world's production of coffee is provided by *Coffea arabica*, about 25% by *Coffea canephora*, and less than 1% by *Coffea liberica* and others(Sharma, 2020). The main constituents of coffee are caffeine, tannin, fixed oil, carbohydrates and proteins.It contains 2-3% caffeine,3-5% tannins,13% proteins and 10-15% fixed oils.In the seeds, caffeine is present as a salt of chlorogenic acid (CGA).Also it contains oil and wax.In coffee pulp, condensed tannins are the major phenolic compounds, while in the seeds, phenolic compounds exist primarily as a family of

esters formed between hydroxycinnamic acids and quinic acid, collectively recognized as chlorogenic acids (CGA).

Nigella sativa is an annual flowering plant.This plant is known by numerous names,for example black cumin(English),black caraway seeds (USA), shoniaz (Persian) and kalajira(Bengali) (Khan & Akram Khan, 1999).Nigella sativa(Black Cumin) seeds have wide therapeutic effects and have been reported to have significant effects against many ailments such as skin diseases, jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic hemorrhage, paralysis, amenorrhea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema(Forouzanfar et al., 2014). 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 therapeutical 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(Samarakoon et al., 2010).Black cumin seeds have saponin and alpha hederin 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 (Forouzanfar et al., 2014). The major merits of herbal medicine seem to be their perceived efficacy, long term treatment with lesser efficacy. The present study was conducted to evaluate the anti-inflammatory effect of oregano, black cumin and coffee extract which could be used as insitu gel for treating oral lesions.

Materials and Methods

Materials used

The materials used in this study includes extracted compounds of oregano, coffee and black cumin, these materials were acquired from authentic biomaterial sellers.About 1g of oregano, coffee and black cumin extracts are mixed with 100 mL of distilled water and boiled for 15 minutes, filtered and again concentrated till 10mL(fig 1).The extracts were subjected to antiinflammatory testing using Bovine Serum Albumin assay.

Antiinflammatory testing

The extracts of Oregano, coffee and black cumin are subjected to Bovine serum albumin assay (BSA). The solution containing Oregano, coffee and black cumin formulations are taken in 5 different concentrations of 10 µL, 20 µL, 30 µL, 40 µL, 50 µL in solution containing 1mL of BSA, 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 for inhibition level analysis.

Result

The results of this study has shown that the formulation of Oregano,Coffee and black cumin has a better Inhibition action in anti-inflammatory assay analysis.

Spectrophotometry readings of anti-inflammatory assay have revealed that formulation of Oregano,coffee and Black cumin of about 10 ml has given an absorption percentage of about 65%when compared with standard . The anti-inflammatory assay has revealed that 75% of inhibition percentage has seen in 40µl &50µl concentrations of Oregano, coffee and black cumin when compared to other levels (Fig.2).

Discussion

Natural compounds with varying mechanisms of action may be used to treat inflammatory diseases. Oregano and other herbs provide anti-inflammatory activity. According to one literature review, the oils and constituents of oregano oil, such as thymol and rosmarinic acid, appear to have anti-inflammatory properties. Dietary antioxidants help the body eliminate free radicals, which are toxic substances that result from natural processes and environmental stresses. A buildup of free radicals can trigger oxidative stress. Oxidative stress can lead to cell damage that may result in various diseases, including cancer and diabetes. Polyphenols are well known natural products known to possess several notable biological propertiesIn a previous work,the presence of five major phenolic compounds with antioxidant properties in hexane and methanol extracts of Oregano was reported[6].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.The major constituents of coffee bean are an alkaloid caffeine, polyphenolic compounds like tannins and a phenolic acid namely chlorogenic acid[13].Sangita *et al.*, concluded that coffee possessed marked invitro anti-inflammatory effect against the denaturation of protein. The effect was possibly due to the polyphenols contents of coffee.Coffee also contains two specific diterpenes,cafestol and kahweol,which have anticarcinogenic properties[16].Nigella sativa extract showed antibacterial synergism 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[9]. 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 [10]. Black cumin studies in recent years for the treatment of microbial disease has been used without any reported side effects.Caffeine has also been proposed as the component of coffee linked to the increased risk in women, with potential influence on calcium absorption and bone mineral density.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.Previous studies have consistently found that long-term coffee consumption is associated with a lower risk of Parkinson's disease(Poole et al., 2017).

Previous studies have proposed that the best form to administrate *O. vulgare* extracts to maintain the antioxidant properties is the encapsulated form, that is, two capsules of 250 mg of a hydroalcoholic extract of *O. vulgare* with a minimum of 33 % of rosmarinic acid as a daily dose[7].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, with summary estimates indicating largest reduction in relative risk at three cups a day(Grosso et al., 2016).A meta-analysis showed a lower incidence of cancer for high versus low coffee consumption(Yu et al., 2011).High versus low coffee consumption was associated with a lower risk of prostate cancer(Wang et al., 2016), endometrial cancer(Godos et al., 2017), melanoma(Godos et al., 2017; Gong et al., 2016),oral cancer(Wang et al., 2016), leukaemia(Yu et al., 2011), non-melanoma skin cancer(Caini et al., 2017),and liver cancer(Bravi et al., 2017) there were also significant linear dose-response relations indicating benefit(Poole et al., 2017).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 (Ludwig et al., 2014).Coffee consumption appears to have a protective benefit in oral cancer(Li et al., 2016).Tavani et al found that different coffee beverages (caffeinated, decaffeinated and coffee) have different effects on oral cancer risk(Tavani et al., 2003).

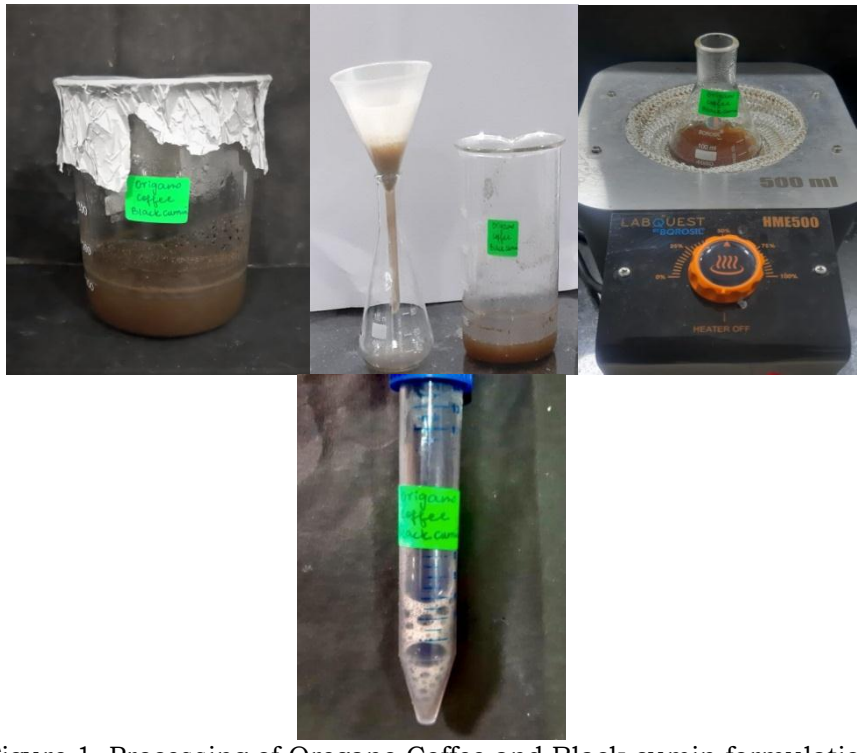

Figure 1. Processing of Oregano,Coffee and Black cumin formulation

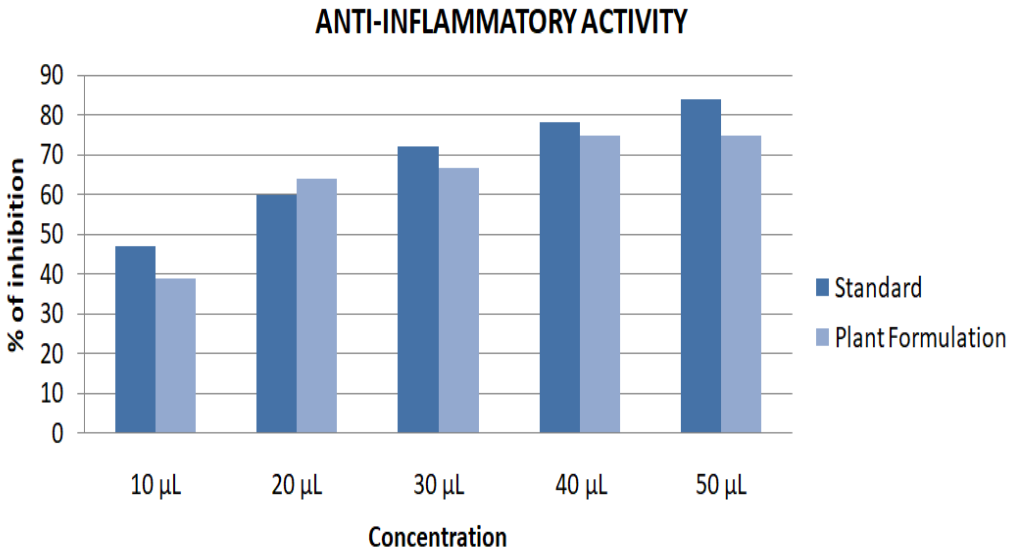

Figure 2. Represents the percentage of inhibition in anti-inflammatory assay seen in levels of 10µl to 50µl concentrations of Oregano, coffee and black cumin formulations

Conclusion

The present study was done to evaluate antiinflammatory property using modern technique, and spectrometry analysis and this study concludes that combination of Oregano,c offee and black cumin formulation has better anti-inflammatory properties, and in future this can be used in the form of in-situ gel in the management of Oral Potentially Malignant disorders such as Leukoplakia, Lichen planus and Oral submucous fibrosis.

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