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RESEARCH ARTICLE

In-vitro efficacy analysis of aqueous extract of *Zingiber officinale, Boswellia serrata* and *Coleus aromaticus* against pathogenic *Staphylococcus aureus*

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Abstract

In the present study aqueous extracts of *Zingiber officinale*, *Boswellia serrata* and *Coleus aromaticus* were investigated for the presence of various phytochemicals. Their antioxidant and antimicrobial activities were also determined against *Staphylococcus aureus*. Phytochemical analyses revealed the presence of phenols, flavonoids, terpenoids, proteins, carbohydrates and saponins in different extracts. The susceptibility of bacterial strain against the aqueous extracts was determined using the well diffusion method. The zone of inhibition of different concentrations of the aqueous extracts against microbe was found to be in the range of 7-27 mm. The results showed that the aqueous extract taken in the concentrations of 50µg/ml (*Coleus aromaticus*), 100µg/ml (*Boswellia serrata*) and 200µg/ml (*Zingiber officinale*) were effective in antimicrobial activity against the test pathogen. Highest antibacterial activity was observed with *Coleus aromaticus* extract with 27 mm diameter of zone of inhibition while minimum activity was observed with aqueous extract of *Zingiber officinale*. The antioxidant activity was revealed by reducing power assay method and showed that aqueous extract of *Coleus aromaticus* has good reducing power which leads to potential antioxidant activity.

Keywords: phytochemicals, antimicrobial, antioxidant

INTRODUCTION

Nosocomial infections pose a significant threat to patients worldwide. Gram-positive bacteria pathogens are a significant reason of nosocomial infections that are important causes of morbidity and mortality (Tacconelli, 2008). The use of plants as medicine is an ancient practice common to all societies. However the knowledge of the appropriate of medicinal application of the plants is still a bit mysterious. It has been estimated that there are 250,000 to 500,000 thousand species of plants on earth (Borris, 1996). Relatively small percentage (1 to 10 %) of these is used as food for both human and other animals (Moerman, 1996). In recent years antimicrobials derived from the plants have been receiving increasing attention, as synthetic antimicrobials have shown ineffectiveness against several pathogenic organisms due to development of drug resistance. Antimicrobial activity has been reported in several plant constituents such as phenols, quinines, flavones, flavonoinds, flavonol, tannins, terpenoids, essential oils and alkaloid etc (Harborne, 2000).

Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last three decades. This has attracted a great deal of research interest in natural antioxidants. Several herbs and spices have been reported to exhibit antioxidant activity, ginger, and several medicinal plants extracts. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, β-carotene, and α-tocopherol are known to possess antioxidant potential (Halliwell, 1998).

The aim of this study was to determine the phytochemical composition, antioxidant activity and antimicrobial activity for aqueous extract of *Zingiber officinale, Boswellia serrata* and *Coleus aromaticus* by investigating their effects on inhibition of biological activity of gram positive bacteria, *Staphylococcus aureus.*

Materials and Methods

Preparation of plant extract

The fresh leaves of test plants were collected from the local area of Gwalior, Madhya Pradesh, INDIA. The leaves were thoroughly washed with tap water followed by distill water and were shade dried. Dried leaves were crushed with the help of mortar and pestle and a fine powder was prepared.

10 g of powdered sample was dissolved in 100 ml of distilled water and boiled for 2 h on slow heat. The residue was removed by filtering through 8 layers of muslin cloth; the filtrate was then centrifuged at 5000g for 10 min. The supernatant was collected and further boiled till the volume was reduced to one fourth of the original volume of the solvent used giving the concentration of 400 µg/ml (Harborne, 1973). It was then autoclaved at 121^0C and at 15 lbs pressure and stored at 4^0C.

The test organism

The test organism was isolated from pus samples obtained from local pathology. The samples were processed by standard microbiological culture practices. The isolates were subjected to Gram's staining and other biochemical tests according to standard procedures. The culture biochemically identified as *Staphylococcus aureus* was selected as the test organism for the further study.

Screening of Phytochemical Compounds

The aqueous extract of *Zingiber officinale, Boswellia serrata* and *Coleus aromaticus* was subjected to phytochemical tests for the identification of various active constituents, using the following methodology:

Detection of flavonoids

To 1 ml of the plant extract, 200 µl of alcohol was added with a few drops of neutral ferric chloride solution. Formation of blackish red colour indicates the presence of flavonoids.

Detection of phenols

To 1 ml of the plant extract, 5 ml of alcohol and a few drops of neutral ferric chloride solution were added. The change in colour indicates the presence of phenols.

Detection of protein

To 5 ml of plant extract, 5ml of distilled water was added and were subjected to Ninhydrin Test. Change in colour indicates the presence of protein.

Detection of saponins

To 1 ml of the plant extract, 20 ml of distilled water was added and then agitated in a graduated cylinder for 15 min. The formation of foam indicates the presence of saponins.

Detection of sugars

To Five ml of the plant extracts, distilled water was added, filtered and then subjected to Fehling's test. A small portion of the various filtrates was treated with 1 ml of Fehling's solution I and II and then heated gently. The formation of reddish brown colour indicates the presence of sugars.

Antimicrobial study

Antibacterial activity was assayed by well diffusion method. Overnight culture grown in broth was adjusted to 0.5 McFarland's density. Media was spread onto 20 ml of sterile agar plates by using a sterile cotton swab. The surface of the medium was allowed to dry for about 3 min. Test plant extracts were dissolved in water to yield the final concentration (50, 100, 150, 200 and 250 µg/ml). Distilled water was used as control. 60µl of sample aliquots were pipette in to the well. The plates were then incubated at 37 ºC for 24 hrs for bacteria after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using zone of inhibition measuring scale. The end of inhibition is where the growth started.

Antioxidant activity

Reducing power of *Zingiber officinale, Boswellia serrata* and *Coleus aromaticus* extracts was determined by the method of Oyaizu, 1986. Different concentrations of *Zingiber officinale, Boswellia serrata* and *Coleus aromaticus* extracts (50-250 µg/mL) in 1mL of distilled water were mixed with phosphate buffer (1 mL, pH 6.6) and potassium ferricyanide (1mL, 1%). The mixture was incubated at 50ºC for 20min. 1mL of trichloroacetic acid (10%) was added to the mixture which was then centrifuged for 10min at 3000 rpm, the upper layer of the solution (supernatant, 1.5 mL) was mixed with D/W (1.5mL) and ferric chloride (100µL) and then incubated for 10min. The absorbance was measured at 700nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Result and Discussion

In view of several drawbacks of synthetic compounds, preparations of plant origin have received escalating attention. There is an increased quest to obtain natural antioxidants and antimicrobials with broad-spectrum actions. The majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. Furthermore, the relationship between phenol content and antioxidant activity is largely not examined in Indian medicinal plant. So, this study was a preliminary attempt in validating this relationship which showed positive results as well.

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc. and getting these chemicals out into the herbal remedy depends upon the solubility of these compounds in various solvents. The effectiveness of the plant is not due to one main active constituent but due to a combined action of other secondary metabolites (Prior, 2003; Cai et. al., 2004). Research is taking place in the hope of finding out effective antimicrobials, to treat human infections. Since the screening methodology for detection of such agents, their isolation from the plants and successive structure activity study are easy to perform there are grounds for optimism in the field of herbal medicine (Landbo & Meyer, 2010).

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as by-products of biological reactions or from exogenous factors. *In vivo*, some of these ROS play positive roles in cell physiology; however, they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer, degenerative, and other diseases (Shanmugam, 2013).

Mammalian cells possess elaborate defense mechanisms for radical detoxification. Key metabolic products are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, non enzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α- tocopherol, β-carotene, and ascorbic acid. Thus, plants can be a good source of exogenous antioxidants (Deiana, 1999; Lee & Shibamoto, 2000; Wang & Jiao, 2000).

Therapeutic potential of plants is mainly due to the presence of bioactive compounds. Among different bioactive compounds poly phenols are antioxidants responsible for the prevention of chronic diseases and health care (Sati *et al*., 2010). A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported in several studies. Many Indian medicinal plants are considered potential sources of antioxidant compounds. In some cases, their active constituents are known while majority of plants are still to be analysed for their phytoconstituents and therapeutic potentials.

In the present study, the phytochemical analysis revealed that the aqueous extracts of *Zingiber officinale*, *Boswellia serrata* and *Coleus aromaticus* possess phenol, proteins, saponins and sugars. Flavonoid and terpenoids were present in *Boswellia serrata*, *Coleus aromaticus* possess flavonoid and *Zingiber officinale* plant extract lack both these phytoconstituents (Table no. 1).

The experiments were done to study the antimicrobial activity of aqueous extracts against the test pathogenic microbe, *Staphylococcus aureus*. The concentration chosen were 25, 50, 100 and 200µg/ml. The experiment was performed using well diffusion method. Antibacterial activity obtained in this study varied with plants used for study (Table no. 2)

Z. officinale extracts showed moderate inhibition activity with the zone range of 7–14 mm. A higher zone of inhibition was observed in 200µg/ml of aqueous leaf extracts. Malu *et al*. (2009) reported anti bacterial activity of various extracts of *Z. officinale* against *C. bacillus*, *S. epidermidis* and *S. viridians* (Polasa & Nirmala, 2003; Kaushik *et al*., 2014). *Z. officinale* is known to contain resins and volatile oils such as borneol, camphene, citral, eucalyptol, linalool, phenllandrene, zingiberine and zingiberol phenols which may be responsible for its potent antimicrobial activities (O'Hara *et al*, 1998).

Boswellia serrata extracts showed good antibacterial activity with the zone range of 8-22mm. A higher zone of inhibition was observed in 100µg/ml of aqueous leaf extracts. The resinous part of *Boswellia serrata* contains monoterpenes (α-thujene); diterpenes (macrocyclic diterpenoids such as incensole, incensole oxide, iso-incensole oxide, a diterpene alcohol [serratol]); triterpenes (such as α- and β-amyrins); pentacyclic triterpenic acids (boswellic acids); tetracyclic triterpenic acids (tirucall-8,24-dien-21-oic acids) (Siddiqui, 2000). Antimicrobial effects of *B. serrata* have not been reported so far. Boswellia has been used in traditional medicine for the treatment of thrush that is caused by Candida species (Weckesser, 2007).

Coleus aromaticus extract showed best antibacterial activity among the tested plant extracts with the zone of inhibition range of 19-27mm. A higher zone of inhibition was observed in 50µg/ml of aqueous leaf extracts. The morphology, phytochemistry and pharmacological aspects of Coleus have been reported. The essential oil from the arial parts of *C. aromaticus* contained twenty six compounds. These compounds were of variable percentages, the major constituent was thymol (63.5%), followed by terpinene (11.8 %), cymol (7.7 %) and caryophyllene (7.1 %) (Weli *et al*., 2011). Other studies of this plant showed variations in percentage of the compounds such as Mallavarapu and coworkers identified carvacol (67%) as the major constituent (Mallavarapu, 1999), but Baslas and colleagues found thymol (41%) to be the main compound of the species growing in India (Baslas & Kumar, 1981).

Antioxidant activity assessment may require a combination of different methods, and it is difficult to assess the antioxidant activity of spices on the basis of a single method. However, the results obtained using the reducing power assay method to evaluate the antioxidant activity showed that the aqueous extract used in the present study can be considered good sources of natural compounds with significant antioxidant activity.

The ability of antioxidant efficiency of the extracts can be quantified using spectrophotometer. The extent of scavenging causes a proportionate change in the absorption. The presence of reducers (the antioxidants) causes the conversion of the Fe^{3+} /ferricyanide complex to the ferrous form (Gulcin *et al*., 2003). The formation of Perl's Prussian blue indicates a higher reducing power. It was reported that, the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada *et al*., 1992).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity, increased absorption of the reaction mixture indicates increased reducing power (Meir *et al*., 1995). The aqueous extract of Coleus showed highest absorbance in comparison to the other extracts involved in the study. Accordingly, the aqueous extract of *Coleus aromaticus* might contain higher amount of reductone, which could react with free radicals to stabilize and block radical chain reactions.

Our results suggested that test plant extracts included in the study can serve as potential source of bioactive healthy compounds in the diet and its consumption could be useful in the prevention of diseases. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use. From the obtained results of our present study we can indicate that plant extracts have exhibited good antimicrobial properties and significant natural antioxidant activity. Therefore, consumption of the plant material might be helpful in combating the progression of various diseases with oxidative stress components such as atherosclerosis, diabetes mellitus and to fight against the microbial infections.

Table No. 1. Phytochemical analysis of test plants in aqueous solvent

Extract	Flavonoid	Phenol	Protein	Saponin	Terpenoids	Sugars
Zingiber officinale	-	+	+	+	-	+
Boswellia serrata	+	+	+	+	+	+
Coleus aromaticus	+	+	+	+	+	+

(+) presence of compounds, (-ve) absence of compounds

Table no. 2. Sensitivity of the test microbe *Staphylococcus aureus* to different concentrations of aqueous extracts of test plants

S. No.	Plant extract	Zone of inhibition (in mm)				
		Extract concentration (µg/ml)				
		50	100	150	200	250
1	*Zingiber officinale*	0	7	9	12	14
2	*Boswelia serrata*	8	14	16	18	22
3	*Coleus aromaticus*	19	21	22	24	27

The extracts showed inhibitory activity producing zone of inhibition in growth. Isolates with zone of inhibition diameter less than 10 mm were considered to be resistant while isolates with diameter more than 10 mm were sensitive to the respective extract concentration. MIC of the extract was that minimum concentration at which the growth was inhibited.

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