

Pharmacognostical evaluation and antibacterial activity of medicinally important spices occurred in local area of Manipur

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ABSTRACT

Many diseases are associated with oxidative stress caused by free radical. Plants are rich in variety of bioactive compounds in the form of primary metabolites and secondary metabolites including aromatic substance, most of which are phenols or their oxygen substituted derivative (such as tannin). These compounds possess high antioxidant properties. Spices have been known not only for flavouring, colorant and preservative but also being used for their potential benefit. In the present study, *Zanthoxylum armatum*, *Allium odorum* and *Eryngium foetidum* are taken into consideration to investigate the phytochemical constituent and antibacterial properties in their ethanol and aqueous extract. The result showed the presence of alkaloids, tannins, saponins, phenols, flavonoids and volatile oil that are common to all the ethanol extract. The antimicrobial activity of these solvent extract was test against two gram positive bacteria (*Staphylococcus aureus*: ATCC-11632 and *Bacillus cereus*: ATCC-10876) and two gram negative bacteria (*Klebsiella pneumonia*: ATCC-10031 and *Pseudomonas aurogenosa*: ATCC-15442) by agar well diffusion method. In both ethanol and aqueous extract of *Zanthoxylum armatum* showed maximum antibacterial activity.

Keywords: Spices, Photochemical and antibacterial activity.

INTRODUCTION

Plant biodiversity represents the primary source for food, feed, shelter, medicines and many other products and many life on earth possible and enjoyable. With ever increasing population pressure and fast depletion of natural resources, it has become extremely important to diversify the present-day agriculture in order to meet various human needs ^[1]. Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in bioactive phytochemical constituents that produce specific physiological action on the human body ^[2]. The phytochemical bioactive compounds are in the form of primary metabolites and secondary metabolites including aromatic substance, most of which are phenols or their oxygen substituted derivative (such as tannin). These compounds possess high antioxidant properties^[4]. They are the basic source for the establishment of several pharmaceutical industries since they have enormous medicinal properties. Spices valued commodities contribute not only flavours but also serve as colorants and preservatives in a wide variety of cultures. Today, spices are increasingly revered not only for their culinary properties but also for their potential health benefits. Although the health attributes associated with spice use may arise from their antioxidant properties, their biological effects may arise from their ability to induce changes in a number of cellular processes.^{[3][4]}

MATERIALS AND METHODS

Three plant materials (*Zanthoxylum armatum*, *Allium odorum*, *Eryngium foetidum*) were collected from various valley district of Manipur. Leaves were removed, washed with water and dried under the shadow for 1-2 week. The

powdered leaf samples were subjected to successive extraction with ethanol and distilled water using Maceration Method.^{[5]-[12]}

Procedure:

1. **Test for alkaloids (Hager's Test):** 1ml of each extract was treated with 1 ml of Hager's reagent (saturated picric acid solution). Formation of orange colour precipitate indicates the presence of alkaloids.
2. **Test for flavonoid (Alkaline Reagent Test):** 1ml of NaOH solution was added in 3ml of each extract. Formation of intense yellow colour indicates the presence of Flavonoid.
3. **Test for Saponins (frothing test):** 1ml of each extract was diluted with 2ml of distilled water. The mixture was then kept in boiling water bath for boiling and existence of froth formation during warming confirms the presence of saponins.
4. **Test for Tannins (Ferric chloride test):** 1ml of each extract was added with 5ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this a few drops 0.1% FeCl₃ solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.
5. **Test for Phenols (Ferric chloride test):** 3ml of each extract was added with few drops of neutral 0.5% FeCl₃ solution. Formation of dark green colour indicates the presence of the phenolic compounds.
6. **Test for Cardiac Glycosides (Keller- Mililani's test):** In 2ml of each extract was added with 2ml of 3.5% FeCl₃ solution and was allowed to stand for a minute. About 1ml of conc. H₂SO₄ was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence of cardiac glycosides.
7. **Test for Steroids (Salkowski):** 5ml of each extract was dissolved in 3ml of chloroform. To this mixture, 2ml of H₂SO₄ was added carefully to form lower layer. A reddish-brown color at the interface indicated the presence of steroids.
8. **Test for Terpenoids:** 5ml of each extract was added with 2ml of chloroform. It was followed by the addition of 3ml of conc. H₂SO₄. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids
9. **Test for Volatile Oils:** 1ml of each extract was mixed with diluted HCl. A white precipitate that may be formed indicated the presence of volatile oil
10. **Detection of carbohydrates (Benedict's test):** 5ml of each extract were treated with 3-10 drops of Benedict's reagent and heated gently for 8-10mins. Orange red precipitate indicates the presence of reducing sugars.
11. **Detection of proteins and amino acids (Ninhydrin Test):** 2ml of each extract was added with 0.5ml of ninhydrin reagent and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Antibacterial activity:

Each extract was tested for antimicrobial activity by agar well diffusion method against different pathogenic microorganisms; two gram positive bacteria (*Staphylococcus aureus* ATCC-11632 and *Bacillus cereus* ATCC-10876) and two gram negative bacteria (*Klebsiella pneumonia* ATCC-10031 and *Pseudomonas aurogenosa* ATCC-15442). Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 5 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, different concentrations (10, 20, 30, 40 and 50µg/ml) of solution was poured onto each well on all plates. After incubation at 37 °C for 24 h, the diameter of zone inhibition was measured in millimetre.

RESULTS AND DISCUSSION

In the present investigation, preliminary phytochemical screening has been done in the various extracts of *Zanthoxylum armatum*, *Allium odorum*, *Eryngium foetidum* showed the presence of phytochemical constituents namely alkaloids, flavonoids, glycosides, saponins, steroids, tannins, phenols, terpenoid, volatile oils and amino acids (Table no.1). ethanol extract of *Zanthoxylum armatum* showed maximum antibacterial activity follows *Allium odorum* and *Eryngium foetedum* respectively and in aqueous extract, *Zanthoxylum armatum* showed large zone of inhibition against *S. Aureus*. MIC values of ethanol extract and aqueous extract are showed in fig no.1.

Table - 1: Phytochemical analysis of Selective Manipuri culinary spices

Phytochemical	<i>Zanthoxylum armatum</i>		<i>Allium odorum</i>		<i>Eryngium foetidum</i>	
	Ethanol	Water	Ethanol	Water	Ethanol	Water
Alkaloids	+	+	-	-	+	-
Flavonoids	-	+	-	+	+	+
Saponins	+	-	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Cardial glycosides	+	-	-	-	+	-
Steroids	+	+	-	-	-	-
Terpenoid	+	+	-	-	+	+
Volatile oils	+	+	+	+	+	+
Carbohydrates	-	-	-	-	-	-
Amino Acids	-	-	+	+	-	-

+ = presence; - = absence

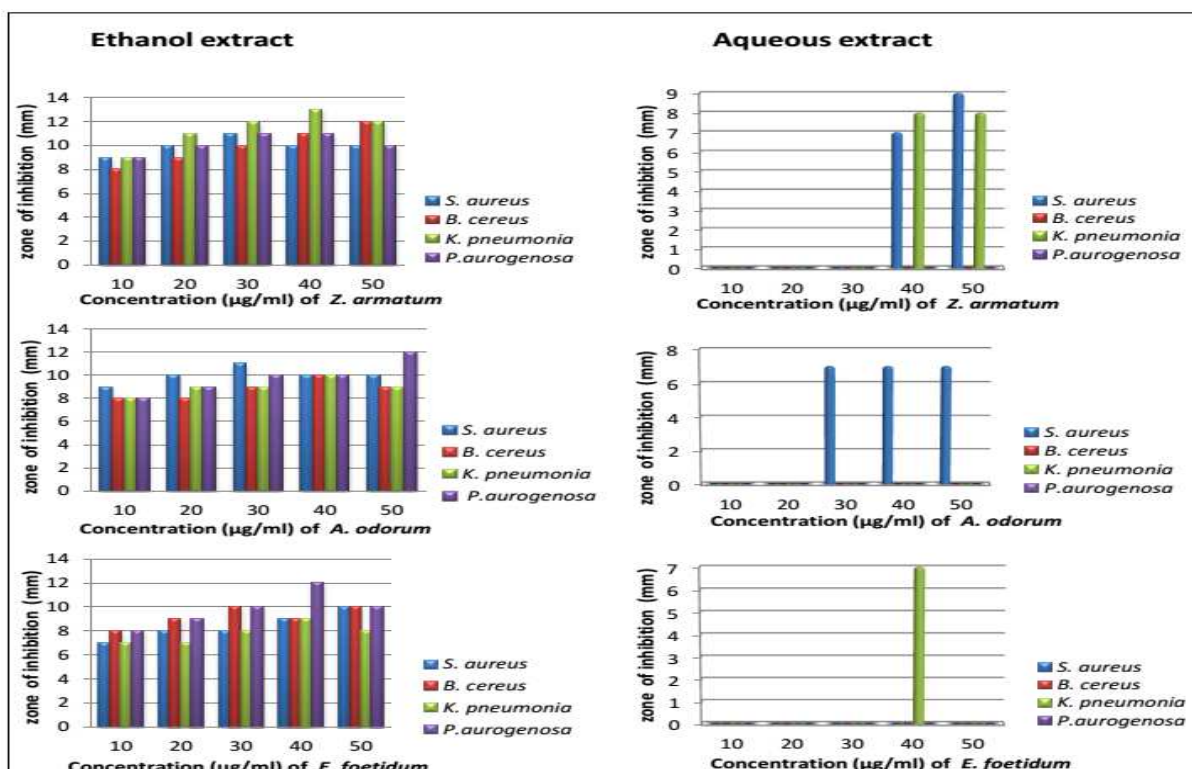


Fig no.1:- MIC values of spice extracts in different solvent (ethanol and aqueous solvent)

CONCLUSION

The traditional medicine all over the world is nowadays revalues by an extensive activity of research on different plant species and their therapeutic principles and nature which they posses. However, further study is necessary to quantify, isolate, characterize and to evaluate biological activity of the particular compound for drug development.

Acknowledgement

The authors are thankful to DST, New Delhi, India and Coordinating Agency: NASI, Allahabad, India for the financial assistance of the present study to the Institutional Biotech Hub, Department of Biotechnology, S. Kula Women’s college, Nambol, Manipur.

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