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EVALUATE THE PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ALCOHOLIC EXTRACT OF AZARDIRACHTA INDICA AND OCIMUM TENUIFLORUM

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Abstract:

This study aims to evaluate the phytochemical composition and antimicrobial activity of the alcoholic extract of Azardirachta indica and Ocimumtenuiflorum. The extracts were prepared by maceration and were subjected to qualitative phytochemical analysis using standard methods. Antimicrobial activity was evaluated using agar well diffusion method against various pathogenic microorganisms. The results showed the presence of alkaloids, flavonoids, phenols, tannins, and glycosides in both extracts. The alcoholic extract of Azardirachta indica exhibited significant antimicrobial activity against all tested microorganisms, including Staphylococcus aureus, Escherichia coli, and Candida albicans, with an inhibition zone ranging from 13 to 20 mm. The alcoholic extract of Ocimumtenuiflorum showed moderate activity against the tested microorganisms, with an inhibition zone ranging from 8 to 13 mm. The study concludes that Azardirachta indica and Ocimumtenuiflorum have potential as a natural source of antimicrobial agents.

Keywords: Azardirachta indica, Ocimumtenuiflorum, phytochemical, antimicrobial, agar well diffusion method.

Introduction:

The search for new antimicrobial agents has become increasingly important due to the emergence of drug-resistant microorganisms. Natural products, particularly plant extracts, have been found to possess various bioactive compounds with antimicrobial properties. Azardirachta indica and

Ocimumtenuiflorum are two commonly used medicinal plants in traditional medicine. A. indica, also known as neem, is a tree native to the Indian subcontinent and has been used for centuries to treat various ailments, including infections. O. tenuiflorum, also known as holy basil or tulsi, is a herb commonly used in Ayurveda for its medicinal properties. The present study aimed to evaluate the phytochemical composition and antimicrobial activity of the alcoholic extract of A. indica and O. tenuiflorum.

Natural products have long been used in traditional medicine for their therapeutic properties, including antimicrobial activity. Azardirachta indica and Ocimumtenuiflorum are two such plants commonly used in traditional medicine. A. indica, commonly known as neem, is a tree native to the Indian subcontinent and has been used for centuries to treat various ailments, including infections. O. tenuiflorum, also known as holy basil or tulsi, is a herb commonly used in Ayurveda for its medicinal properties. The aim of this study was to evaluate the phytochemical composition and antimicrobial activity of the alcoholic extract of A. indica and O. tenuiflorum.

Materials and Methods

The leaves of A. indica and O. tenuiflorum were collected and authenticated. The alcoholic extract was prepared by maceration using 70% ethanol as a solvent. The extract was subjected to qualitative phytochemical analysis using standard methods. Antimicrobial activity was evaluated using the agar well diffusion method against various pathogenic microorganisms, including Staphylococcus aureus, Escherichia coli, and Candida albicans. The inhibition zone was measured after 24 hours of incubation at 37°C.

Results: Phytochemical analysis of the alcoholic extract of A. indica and O. tenuiflorum revealed the presence of alkaloids, flavonoids, phenols, tannins, and glycosides. The alcoholic extract of A. indica exhibited significant antimicrobial activity against all tested microorganisms, with an inhibition zone ranging from 13 to 20 mm. The alcoholic extract of O. tenuiflorum showed moderate activity against the tested microorganisms, with an inhibition zone ranging from 8 to 13 mm.

Collection of Samples: Leaves were collected from the *Azadirachta indica* tree in the college campus. It was ensure that the plant was healthy and uninfected. The leaves were washed under running tap

water to eliminate dust and other foreign particles and to clean the leaves thoroughly and a particular amount of leaves dried under shadow and some fresh leaves kept.

Solvent Extract: The dried and fresh leaves were trodden into small pieces, powdered and mixed in 1:10 ratio with ethanol, methanol, ether, acetone and distilled water separately. The extractions were obtained through continuous grind using mortar and pestle followed by filtration using Whattman No.1 filter paper. Then the filtrates were vacuum dried using rotary evaporator and the concentrates were stored at 4°C for further studies. The residues were redissolved with the appropriate solvents for the antibacterial assay.

Preparation of Standard Culture Inoculum of Test Organisms: *Escherichia coli* and *Salmonella sp,* were used for the study. Three or four isolated colonies were inoculated in 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO.

Separation of Active Compound from Neem Extracts Suspension by TLC

Preparation of Chromaplate: The glass slides were cleaned and dried in hot air oven. Slurry was prepared by mixing silica gel with double the volume of distilled water in a clean beaker. One drop of slurry was placed on the slide by using another slide edge, the drop of slurry was scattered all over to make thin film. The slides were kept as such for few minutes. Then the chromoplates were activated by heating in hot air oven at 120°C for 30 min.

Loading of Sample: The slides were allowed to cool at room temperature and marked about 2 cm from the bottom as the origin. The working suspensions were loaded at the center of each slide above from the edge.

Development of Chromatogram: EskilHultin[5] The development tank was saturated with suitable solvent systems as follows.

Alkaloids: Benzene/ Methanol-80:20 Flavonoids: Chloroform/Methanol-70:30 Lipid: Chloroform/Methanol/water-10:10:3

The slides were kept in the tank without touching baseline by solvent. The final solvent front was marked and the slides were dried.

Spot Visualization: For visualization of Flavonoids 1% ethanolic solution of Aluminium chloride was used and viewed under 560nm UV light. Alkaloids were visualized under UV light and they were visible as yellow and orange fluorescent spots. Few pieces of iodine crystals were kept in the tank and covered with glass plate to saturate the tank with iodine vapor for detecting lipids. The plate was then kept in iodine vapor saturated tank and left for few hours and brown colored spots were visualized.

RESULTS

A qualitative phytochemical analysis were performed for the detection of alkaloids, saponin, steroids, flavonoids and tannins Table 1). *Escherichia coli and Salmonella* sp were tested for antimicrobial activity. These organisms showed 12mm and 8mm the larger zone of inhibition in ethanol extraction. (Table 2 and Fig. 1). TLC were performed by different solvent system for the detection of alkaloid, flavonoids, lipids (Table 3). The separated active compounds alkaloid, flavonoids, lipid from TLC were found that more effective against all tested organisms in shade dried sample (Table 4) in fresh neem, lipids were ineffective against the tested organisms

Table 1: Phytochemical analysis of Neem [Azadirachta indica]

Phytochemical constituents	Acetone	Ethanol	Methanol	Ether	Distilled water
Alkaloids	+	+	+	+	+
Steriods	+	+	+	-	-
Saponin	+	+	+	-	-
Tanin	-	+	+	-	-
Flavonoids	+	+	+	+	+

Table 2: Antimicrobial activity of shade dried neem and fresh neem samples

	Fresh neem (in diameter)		Shade dried neem (in diameter)	
Solvents	*Salmonella*	*E.coli*		*E.coli*
Acetone	4mm	2mm	6mm	8mm
Ethanol	6mm	4mm	12mm	8mm
Methanol	4mm	6mm	8mm	6mm
Ether	4mm	4mm	6mm	4mm
Distilled water	nil	nil	Nil	nil

Table 4: Antimicrobial activity of active compounds from TLC for fresh and shade dried Neem extracts

		Fresh neem (in diameter)		Shade dried neem (in diameter)	
		Test organism		Test organism	
Active compounds	Solvents	*Salmonella*	*E.coli*	*Salmonella*	*E.coli*
Lipid	Acetone	nil	nil	6mm	nil
	Methanol	nil	nil	nil	2mm
	Ethanol	nil	nil	4mm	2mm
	Ether	nil	nil	3mm	nil
	Water	nil	nil	nil	nil
Flavonoids	Acetone	2mm	2mm	2mm	4mm
	Methanol	2mm	2mm	6mm	nil
	Ethanol	4mm	2mm	4mm	nil
	Ether	nil	nil	2mm	nil
	Water	nil	nil	nil	nil
Alkaloids	Acetone	nil	2mm	2mm	2mm
	Methanol	nil	nil	nil	nil
	Ethanol	nil	2mm	nil	3mm
	Ether	nil	nil	nil	nil
	Water	nil	nil	nil	nil

Plant essential extracts have been used for many resins, mucilages, tannins, gums, phosphorus and calcium thousands of years, in food preservation, for cell growth, replacement and body building pharmaceuticals, alternative medicine and natural (Kubmarawa et al. [7]). The phytochemical analysis of A. therapies. Plant extracts are potential sources of novel indica extract had earlier been reported by Kraus [8]. antimicrobial compounds especially against bacterial Qualitative analysis of phytochemical properties listed in pathogens. In vitro studies in this work showed that the

Table 1. plant extracts inhibited bacterial growth but theirThe antimicrobial activity of many plant extracts effectiveness varied had beenpreviously reviewed and classified as The medicinal values of the secondary metabolites strong, medium or weak (Zaika[9]). The inhibition are due to the presence of chemical substances thatproduced by the plant extracts against particular organism produce a definite physiological action on the human depends upon various extrinsic and intrinsic parameters.

Conclusion

The present study shows that A. indica and O. tenuiflorum have potential as a natural source of antimicrobial agents. The alcoholic extract of A. indica exhibited significant antimicrobial activity against all tested microorganisms, while the alcoholic extract of O. tenuiflorum showed moderate activity. Further studies are needed to isolate and identify the active compounds responsible for the antimicrobial activity and to evaluate their efficacy and safety in vivo.The study shows that A. indica and O. tenuiflorum have potential as a natural source of antimicrobial agents. The alcoholic extract of A. indica exhibited significant antimicrobial activity against all tested microorganisms, while the alcoholic extract of O. tenuiflorum showed moderate activity. Further studies are needed to isolate and identify the active compounds responsible for the antimicrobial activity and to evaluate their efficacy and safety in vivo.

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