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SANTALUM ALBUM;

IN VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF SANTALUM ALBUM AND CYMBOPOGON BY SEQUENTIAL EXTRACTION.

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ABSTRACT: Medicinal plants are good alternate of antibiotics against many bacterial as well as other diseases. Santalum album (Sandal) and Cymbopogon (Lemon grass) are two important medicinal plants whose important components were extracted by sequential extraction from non-polar to polar solvents. The study was aimed at finding antibacterial and radical scavenging potential of Santalum album (Sandal) and Cymbopogon (Lemon grass). **Study Design:** In vitro study. **Setting:** Department of Biochemistry and Molecular Biology, University of Gujrat, Gujrat. **Period:** 12 months. **Material and Methods:** Sequential extracts of Santalum album and Cymbopogon with n-hexane, chloroform, acetone, ethylacetate, ethanol, butanol and water respectively were prepared to evaluate antibacterial activity against Staph aureus (25923), Staph aureus (38541), E.coli (25922), E.coli (35318), Streptococcus pyrogenes (Tc-11-2) and Shigella sonnei (BB-8). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used to assess antioxidant activity. **Results:** Ethanolic and acetone extracts of sandal and lemongrass showed significant inhibtory activity against all seven strains. In case of sandal, acetone extract exhibited highest inhibitory activity against Staph aureus (25923) with 17±2 mm zone of inhibition while ethanolic extract of lemon grass showed highest activity with 16.333 ± 1.154mm zone of inhibition against E.Coli (35318). Other solvents including chloroform, n-hexane, ethyl acetate and butanol also showed considerable antibacterial activity, but water extracts of both plants showed no activity. All polarity based extracts of both plants exhibited antioxidant activity, ethanolic extracts of sandal and lemon grass showed highest radical scavenging activity with 84.366 ±1.504% and 83.766 ±4.272% inhibitions respectively. The minimum antioxidant activity was observed for chloroform extracts of sandal and n-hexane extract of lemongrass. **Conclusion:** we concluded that some plants have good antibacterial and antioxidant potential. Their phytochemical analysis can be carried out to find potent antibacterial and antioxidant compounds. This will be effective in combating bacterial diseases because mostly microbes are developing resistance against currently available antibiotics.

Key words: Antibacterial, Antioxidant, Extracts, Santalum Album.

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INTRODUCTION

Plants are very important source of medicines, for treating many diseases.[1] The world is rich with natural and medicinal plants. About 65% of the world's population uses traditional medicines according to the World Health Organization (WHO) report.[2] Out of 80000 species with remedial properties, only 7000 to 7500 species world-wide are used for their therapeutic values by traditional communities. Many plants, such as sandal and lemongrass have been considered as medicinal plants since ancient times which can be used potentially as natural antimicrobials, antifungal and antioxidants. Active components of these plants are responsible for curing diarrhea, urticaria, eye infections, fever, cold, cough and stomach problem.[3]

The Santalum album L (sandal tree) is used as medicinal plant from many years. The Sandal wood plant is mainly used as coolant, and also used as sedative effect and astringent activity, making it functional as antiseptic in genitourinary and bronchial tracts, diuretic, expectorant and stimulant. In Ayurveda system other uses of sandalwood mentioned, are in remedial of a

variety of other ailments like diarrhea, infection of eye, vomiting, food poisoning, hiccoughs initial phase of pox, swelling of umbilicus and urticaria.[4]

Lemongrass tea has diuretic properties and water retention, making it helpful in individual with high blood pressure. In Nigeria it is used for stomach problem and it is also used in amalgamation with few other plants for efficient treatment of malaria[5] and typhoid.[6] Lemongrass is used to treat acne, oily skin, scabies, headaches, blood circulation problems because of its antimicrobial activity.[7]

Medicinal plants possess scavenging activity, and are natural sources of antioxidants without any toxicity problems. Antioxidative compounds of medicinal plants used to neutralize reactive oxygen species.[8] Antioxidative activity of plants is due to presence of flavonoids in plants.[9]

Resistance of bacteria's to presently available synthetic drugs is becoming a major concern of public health.[10] Therefore, this study was aimed at finding antimicrobial and antioxidant activities of sequential extracts of locally available sandal and lemongrass.

MATERIALS AND METHODS

Collection of Medicinal Plant Material

Both the medicinal plants, sandal heartwood and lemongrass stalk were purchased from local market of Gujrat. The plants were identified by the department of Botany, University of Gujrat.

Bacterial Strains

The test bacteria used were Escherichia coli (25922), E.coli (35318), Staphylococcus aureus (25923), Staphylococcus aureus (38541), Streptococcus pyogenes, neisseria gonorrhoeae and Shigella sonnei. All microorganisms were obtained from the Department of Biochemistry and Molecular biology, University of Gujrat.

Preparation of Plant Extract

The heartwood of sandal and lemongrass were dried and grinded into fine powder. Sequential extraction was carried out by using different solvents (n-hexane, chloroform, acetone, ethylacetate, ethanol, butanol and water respectively) on the basis of their polarity. Samples of both plants were extracted in triplicates. Sample to solvent ratio was 1:10. After overnight shaking, dissolved mixture was filtered, dried and condensed, and residue was dissolved in next solvent and so on based on increasing polarity.

Antimicrobial Activity Assay

Initially, bacterial cultures were refreshed from the glycerol stocks on lb broth medium at 37°C for 24 hours. Next day autoclaved lauria Bertini (LB) agar media was prepared, which then cooled and poured in petri plates under sterilized condition in laminar flowhood, then seven bacterial strains were spreaded in plates and two antibiotics in form of discs were placed in plates, antibiotics used were Ofloxacin (OFX), Imipenem (IPM). Wrapped the plates with parafilm and placed in incubator overnight, next day plates were checked.

Antibacterial activity was checked through disc diffusion method. Test bacteria's stock cultures were grown in lb broth medium at 37°C for 24 hours. After autoclaving, Plates were prepared with 25 ml agar. The lawn culture of seven different bacterial strains was then prepared on (LB) agar. The autoclaved paper discs (5mm) soaked in each of the test solutions containing different fourteen extract solutions of both plants, as well as the standard drugs OFX or IPM were placed separately in each quarter of the plate under aseptic conditions. All petri plates were then incubated at 37°C for 24 hours and antimicrobial activity was assessed in mm on the basis of diameter of zones of inhibition.[11]

Antioxidant Activity of Sandal and Lemongrass

To determine antioxidant activity of sandal and lemongrass 2,2-diphenyl 1- picryl hydrozyl assay used.[12] The antioxidant assay was carried out by adding an aliquot of each extract in 2ml of 0.1mM ethanolic DPPH. After 15 min incubation at room temperature, the absorbance was recorded through spectrophotometer at 517 nm. The same experiment was repeated three times. Ascorbic acid was used as standard.

RESULTS

Antimicrobial activity of Sandal and lemongrass

was exhibited by all extracts except water. Water and acetone extracts of sandal showed maximum percentage yield. But ethanolic and acetone extracts of sandal showed maximum antimicrobial activity this means that percentage yield was not dependant on antimicrobial activity but chief components extracted by acetone and ethanol extracts exhibited activity against bacteria's. On the other hand; acetone, ethanol and butanol extracts of lemongrass exhibited maximum percentage yield and ethanolic extract showed maximum activity. Water extracts of both plants showed significant percentage yield but showed no antimicrobial activity with poor antioxidant activity (Table-I).

In case of Sandal, maximum inhibitory activity was showed by n-hexane extract against Escherichia Coli (25922). Minimum inhibition was against Neisseria gonorrhoeae (4c-11) and Streptococcus pyrogenes (Tc-11-2). Chloroform extract exhibited maximum inhibitory activity against Shigella sonnei (BB-8) and minimum inhibition against Streptococcus pyrogenes (Tc-11-2). Acetone extract showed maximum inhibition with Staph aureus (25923) and minimum inhibition against Streptococcus pyrogenes (Tc-11-2). Ethylacetate extract showed maximum inhibition against Neisseria gonorrhoeae (4c-11) and minimum against Staph aureus (38541). The Ethanolic extract showed maximum inhibitory activity against Staph aureus (25923) and minimum against E.coli (35318). Butanolic extract showed maximum inhibition against Staph aureus (25923) and minimum inhibition activity against E.coli (35318).Water extract showed no activity against any strain of bacterias, because water is highly polar molecule which is miscible only in itself, it has high dielectric constant and dipole moment (Table-II).

In case of lemongrass, n-hexane extract exhibited maximum inhibitory activity against Staph aureus (25923) and minimum inhibition against Streptococcus pyrogenes (Tc-11-2). Chloroform extract exhibited maximum inhibition zone against Staph aureus (25923) and Staph aureus (38541), and minimum inhibitory activity against Neisseria gonorrhoeae (4c-11). Acetone extract showed maximum inhibitory activity against Staph aureus (25923) and minimum inhibition against Neisseria gonorrhoeae (4c-11). Ethyl acetate extract showed maximum inhibition against Streptococcus pyrogenes (Tc-11-2) and showed minimum inhibitory activity against E.coli (35318). Ethanolic extract exhibited maximum inhibition against E.coli (35318) and minimum inhibition against Staph aureus (38541) and Shigella sonnei (BB-8). Butanolic extract exhibited maximum inhibition activity against Staph aureus (25923) and Shigella sonnei (BB-8) and minimum inhibitory activity against E.coli (25922), E.coli (35318) and Streptococcus pyrogenes (Tc-11-2). Water extract of lemon grass showed no activity against any bacterial strain similar to sandal water extract (Table-III).

Acetone and ethanol extracts of sandal and lemongrass exhibited good antioxidant activity but chloroform extract of sandal and n-hexane extract of lemongrass showed poor activities. Acetone extract of both plants showed maximum inhibition because acetone itself has antioxidant activity, n-hexane and chloroform extracts of both plants showed minimum inhibition because flavonoids and phenolic compounds dissolve in polar solvents that showed antioxidant activity and these are non-polar solvents. Water, n-hexane, acetone, butanol and ethyl acetate extract of sandal also exhibited poor to good antioxidant activity, respectively. Similarly, lemongrass chloroform, ethanol, ethylacetate, butanol as well as water extract also showed significant activity (Table-IV).

Test plants	Yield percentage (%)						
	n-Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Butanol	Water
Sandal	4.6	4.67	4.8	6.5	4.9	4.65	6.5
Lemongrass	4.85	4.95	4.9	5.7	5.35	5.45	4.85

Table-I. Percentage yield of Different Solvents Extracts of Sandal and Lemongrass

Bactaria	n-Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Butanol	Water
Staph aureus (25923)	9.334±1.52	9.667±1.527	12±1	17±2	17±1	15±3	-
Staph aureus (38541)	6.66±0.57	9±2	8.33±1.5	10.66±1.15	14.33±2.08	12.66±1.15	-
E.Coli (25922)	13.66±1.52	8.667±0.577	11±1	10.33±0.57	15.66±1.52	12.66±2.51	-
E.Coli (35318)	13.334±1.527	8.66±1.52	11.66±1.52	14±3.60	13.33±3.05	11.66±2.08	-
Streptococcs pyrogenes (Tc-11-2)	6.66±0.57	6.334±0.577	10.33±1.52	10±1	14.66±1.52	12.33±2.51	-
Shigella sonnei(BB-8)	11.66±1.52	11.334±0.577	11.33±1.52	15±3	16.66±1.52	13.33±1.52	-
Neisseria gonorrhoeae (4c-11)	6.33±0.57	8.334±1.154	14.33±1.52	15.66±1.15	16.33±0.57	13.33±1.52	-

Table-II. Antimicrobial Activity of Sandal against Seven Strains of Bacteria

Bactaria	n-Hexane	Chloro-form	Ethyl acetate	Acetone	Ethanol	Butanol	Water
Staph aureus (25923)	9.66± 0.57	10 ± 1	10.33± 1.52	13.33 ±1.52	14.66± 1.52	15 ± 3	-
Staph aureus (38541)	6.66± 0.57	10 ± 1	10.66± 1.52	12 ±1	14 ± 2	14 ± 2	-
E.Coli (25922)	7.33± 0.57	8.33± 1.52	8.66± 1.15	10.66±2.08	15 ± 2	13 ± 2	-
E.Coli (35318)	7.66± 1.52	8.66±1.52	8 ± 1	11.66±1.5	16.33± 1.15	13 ± 1	-
Streptococcs pyrogenes (Tc-11-2)	6.33± 0.57	8.33± 1.52	11 ± 2	11.33±1.52	16 ± 1	13 ± 2	-
Shigella sonnei (BB-8)	7.33± 1.52	9.334± 1.52	10 ± 2	10.33±1.52	14 ± 2	15 ± 1	-
Neisseria gonorrhoeae (4c-11)	8 ± 1	7.66± 1.15	10.33± 2.08	10± 1	15 ± 2	14.33± 2.08	-

Table-III. Antimicrobial Activity of Lemongrass against Seven Strains of Bacteria

Plant Extract	n-Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Butanol	Water
Sandal	28.26±0.25	13.33±1.26	83.03±1.62	78.55±3.37	84.36±1.50	80.60±1.90	25.06±1.75
Lemon grass	22.44±2.82	68.96±0.80	67.26±2.93	89.50±4.10	83.76±4.27	80.60±1.90	42.83±3.45

Table-IV. Antioxidant Activity of Sandal and Lemongrass

DISCUSSION

Different plants are used for the treatment of various diseases since prehistoric times because of their several remedial effects. The use of plants in conventional medicine is still need to be studied, particularly in microbiology. Commercially available antibiotics are mostly derived from microbes.[13] The extraction of biological active compounds mainly depend upon the solvent used in extraction process and most of the active antimicrobial compounds which have been identified are soluble in polar solvent.[14,15]

Ethyl acetate is aprotic solvent with intermediate

polarity and its extract exhibited zone of inhibition against all bacteria's, especially maximum zone of inhibition against Neisseria gonorrhoeae. Ethanolic extract showed maximum zone of inhibition against all seven strains of bacteria and Acetone extract showed almost equal activity as ethanol extracts, because acetone solvent itself also has antimicrobial activity. Ethanolic extract showed maximum because ethanol is a highly polar component.[16] Sandal plant is very rich in polar components than non-polar components, and ethanol has ability to extract maximum polar components from the plant so exhibited maximum activity.

Butanolic extract also exhibited significant activity against microbes but less than ethanolic extracts because most of the polar components were extracted by ethanol and remaining component was dissolved in butanol, so butanolic extract exhibited less inhibitory activity than ethanolic extracts. Water extract showed no activity. It has been already studied; water extracts of various plants exhibited no or less activity.[17]

In sequential extraction of lemongrass n-hexane and chloroform extract showed some activity against microbes, lemongrass consists of mainly polar components and non-polar components. Ethyl acetate is a aprotic polar solvent and it extracts polar components from plants. Acetone also exhibited inhibitory activity against microbes but acetone itself also has antimicrobial activity. This could be because acetone is a dipolar aprotic solvent which means that acetone is of medium polarity; therefore it can dissolve a broad range of compounds[16] but other solvents n-hexane, chloroform, ethyl acetate, ethanol and butanol solvents have no antimicrobial activity. Ethanol and butanol have greater capability to extract polar components from lemongrass plant because of high polarity. Maximum inhibition of bacteria was observed by ethanolic extracts. Water extract showed no activity because of dipole moment, larger size of polar components as well as going from non-polar to polar sequential extraction all the polar and non-polar components was extracted by other solvents.

It is established that reactive oxygen species (ROS) and other oxidants in have key role in causing numerous disorders and diseases. This substantiation has urged the scientists to use antioxidants for prevention and treatment of diseases, and maintenance of human health.[18] Flavonoids, tochopherols and stilbenes are the most important phenolic compounds which are involved in lipid peroxidation and are responsible for antioxidant activity. n-hexane, acetone, ethylacetate, butanol and water extract of sandal exihibited significant antioxidant activity but water extract of sandal exihibited least antioxidant activity, water is highly polar molecule extracted least amount of phenolic compounds from sandal. In case of lemongrass chloroform, ethanol, ethylacetate, butanol and water extract also showed significant activity. It was studied that lemon grass oil has been shown to inhibit the growth of fungi like Aspergillus fumigatus, Candida albicans and Aspergillus niger,[19] Salmonella typhi, Staphylococcus aureus and Escherichia coli.[20]

CONCLUSION

It is concluded that different solvent extracts of sandal and lemongrass have antimicrobial activity against pathogenic bacterias that causes diseases in humans therefore these plants are natural and cheaper source of treating microbial and free redical related diseases such as heart and stomach diseases because these plants also have good antioxidant potential. Comprehensive phytochemical analysis can be carried out to find potent antibacterial components which will be a good approach to combat bacterial diseases.

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2	Sonia Gull	Experimental work, writeup.	
3	Asim Mushtaq	Experimental work, writeup.	
4	Muhammad Umer	Writeup, Proof Reading, Research Plan.