Extraction of Secondary Metabolites from Medicinal Plants and Study of Its Antibacterial Activity Against Normal Micro Flora of the Skin

Arun K. Zingare
Department of Botany, M. B. Patel College Sakoli.
E-mail: arunzingare@yahoo.in

Abstract:
Secondary metabolites are active on the normal microbial flora that can be isolated from crude of medicinal plants leaves. Various kinds of microbial flora present on the surface of skin these are pathogenic as well as non-pathogenic**.** Sometime these microbial floras are susceptible by excretory substance or secondary metabolites such as derivatives of drug.

In methanol extracts of leaves and stems of *Santalum album*, is a plant species of the *Santalaceae*. *Curcuma longa* (Haldi, Ginger) were screened for their antimicrobial activity using the Well-Agar Plate diffusion method. They were tested against isolates of Staphylococcus species like *Staphylococcus aureus, Staphylococcus epidermis;* Gram positive bacteria were present on normal skin.
The susceptibility of the microorganisms to the extracts of these plants was compared with isolates. The antimicrobial activities of these plants were discussed according to their phytochemical components.

Keywords:
Antimicrobial, Gram positive, Medicinal plants; *Curcuma longa,* Santalum *album.*

Introduction:

The chemical process that occurs within living organism to maintain life is called as metabolism. Metabolites are divided into three types, depending upon the role in metabolism and when they are produced. They are Primary metabolites, Intermediary metabolites and Secondary metabolites.
Primary Metabolites produce mainly during metabolism is useful for plant growth. In chemical transformation, which characterization life large numbers of substances are formed, which are immediately subjected to further metabolism.

Secondary metabolites

In secondary metabolites are those chemical compounds in organism that are not directly involved in the normal growth, development or reproduction of organism. Secondary metabolites are often species specific and without these compounds the organism suffer from only mild impairment, lowered survivability. Function or importance of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases. The broad categories of secondary metabolism are classified on their biosynthetic origin. The majority of these

compounds belongs to one of the number of family, each of which have particular structural characteristics arising from the way in which they are built up in nature i.e. from their biosynthesis.

The classes of secondary metabolites are: Polyketes and Fatty acids, Terpenoids and Steroids. Phenyl propanoids, Alkaloids, Specialized amino acids, Specialized carbohydrates. All medicinal plants containing second metabolites can be extracted, purified and studies for a large number of uses including antimicrobial activity.

Sandal Wood Plant

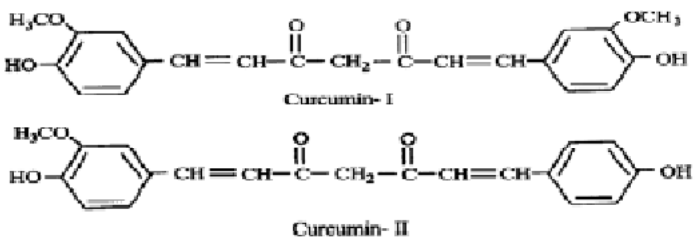
***Santalum album*, Family – *Santalaceae*, Vernacular name: *Sandal wood*.**

Their medium size evergreen semi parasitic, glabrous tree with slender drooping branches, reaching upto 18 m in height; bark dark gray or brownish black, rough with short vertical cracks; leaves simple, opposite elliptic-lanceolate, glabrous, entire. The heartwood is light yellowish brown when fresh, turning dark brown to dark reddish brown on exposure. Santalum leaf consists of dried, mixture leaves from older tree of Santalum album and other preparation in effective dosage. The leaves contain essential oil which consist mainly 1, 8- cineol and tannins. Santalum leaves contain tannins (upto 11%) and associate phenolic acid (caffeis, ferulic, gallic, gentisic and protocatechuic acid), Flavonoid, volatile oil (1.0-3.5%) of which 54–94% is cineole, tritertenes(2-4%), which are ursolic acid derivatives, monoterpenoids, aldehyde (myrtenal) and ketenes (carvone). It is useful in burning sensation, skin diseases, leprosy, jaundice, bronchitis, inflammation, and dysentery. The heartwood is bitter, sweet, acrid, aromatic disinfectant diuretic antipyretic and tonic.

Turmeric Plant

***Curcuma longa*, Family: *Zingaberaceae*, Vernacular name: *Turmaric*.**

Turmeric is Asian plant, *Curcuma longa* (haldi, Ginger). It's height of 5 feet in tropical parts of southern Asia, it has characteristic in having a sharp, bitter taste. Turmeric is used in spice which is obtained from rhizomes of plant *Curcuma longa*, a member of the family Zingaberaceae.



Structural formula of Curcumin (curcuma longa)

Components of turmeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin.

Structural formula of Curcumin (curcuma longa) Turmeric and its major component, curcumin, are both used as phytomedicines (Gislene G F. 2000), coloring agent for curries and other foods, *is* also an important medicinal herb, used by both Chinese and Ayurvedic medicine practitioners (Cikriker S. et al 2008). Latin name curcuma longa or simply curcumin, the Turmeric plant is used to treat a number of medical disorders, including digestive disorders, liver problems, and skin diseases.

Normal Flora of Skin

Most frequently obtained bacteria on the skin are gram +vecocci including *Micrococcus* species and *Staphylococcus* species. *S. aureus* is the part of normal flora of the skin and also considers a pathogen. Sebum secreted from oil gland help the survival of *Propionibacterum acnes,* (Arneja book)

Material and methods:

In the present work normal micro flora of the skin where studied by isolating different microbes from different human parts like face and armpit. These isolates were inoculated into nutrient broth separately for their mass production. To extract anti microbial leaf sample of turmeric and sandalwood were dried, soaked in ethanol and were refluxed using Soxhlet Apparatus. These extract were treated for its anti microbial activity by agar well diffusion method to *microbe's* isolates earlier. Further minimum inhibitory concentrations were determined by observing growth of the isolates *microbes* at 600 nm, in the presence of different concentration of respective plant extracts. Then the comparative studies where made from the result s obtained as seen in agar well diffusion method, O.D. method and were reported accordingly.

Preparation of plant extract

The leaves of the two medicinal plants i.e. *Santalum album* and *Curcuma longa* were sun dried. The sun drying was done for 3-4 days that was followed by grinding. The plant extracts were prepared using the modified method of Alade and Irobi. Briefly, 20 gms portions of dried powdered leaves were soaked separately in 100 ml of ethanol for three days or 72 hours. Then each mixture was refluxed in Soxhlet apparatus, for one hour. This was followed by agitation at 200 rpm for one hour. After this filtration was done.The filtered liquids were kept into the plastic bottles in refrigerator (-19^{0}C) for storage and were further used for antimicrobial activity.

Determination of antimicrobial activity

Isolation of microorganism from normal flora of skin (Face and Armpit). Initially 5 ml of 0.95% saline was prepared in distilled water and dispensed 2.5 ml in each of two sterile test tubes. Two sterile cotton swab were moisture in both the test tube respectively. excess of saline was removed by pushing the swab against the test tube wall then the swab were rubbed over the skin surface i.e. face and armpit respectively. These swabs were again moisture in

the same test tubes respectively and both the tubes were shaken. From each tube 100 ml of saline sample was poured in sterile nutrient agar plates and spreader with glass spreader. Both the plate was then kept from incubation for 24 hours at 37°C. Isolated colonies were obtained after 24 hours and they have been transferred to nutrient agar slants for pure culture. Initially 7 isolated colonies were obtained, but for safety only 3 were proceeds further.

The organisms isolated above were inoculated separately into nutrient broth. They were kept for incubation at 37° C. After 24 hours of incubation the growth of microorganism was seen. About 100 µl of broth containing microorganism was taken as an inoculum for further experiment.

Agar Diffusion assay method

The modified agar well diffusion method to carry out, the nutrient agar prepared. Nutrient agar was autoclaved at 121⁰ C and 15 lb pressure for 15 mins. The media was poured into the previous autoclaved Petri plates into laminar airflow chamber. Once the agar was solidified in the Petri plates, about 0.1ml of the inoculums were poured in to the Petri plates. The spread plate was done for the proper distribution of microorganism into the Petri plates uniformly. After the uniform spreading of inoculums by spreader, it was punched with cork borer creating 0.7 cm diameter wells. Then the concentrations of the different plant extract were taken in the increasing order such as 40 µl, 60 µl, 80 µl, 100 µl, and 120 µl.

These concentrations of each plant extracts were poured into the wells with the help of micropipette and one Petri plates was used as a control for each organism. The above procedure was repeated for both the extracts of the medicinal plant. Then the Petri plates were kept for incubation at 37⁰C for 24 hours. The antimicrobial activity was calculated by measuring the zone of inhibition. Here the diameter was taken into account. The diameter was measured and was reported in results.

Determination of MIC by Turbidometer method

MIC was done to determine the minimum amount of crude extract use to inhibit a particular organism. For MIC sterile test tube containing 5 ml of sterile nutrient broth were required to which particular volume of extract was added. These tubes were then incubated at 37⁰C for 24 hours. All addition were done by using micropipette having sterile tips; during addition of extract and the test isolated. After incubation the O.D. was measure at 600 nm by using UV spectrophotometer. Here, the O.D. was taken into consideration. The O.D. was measured and reported in the result.

Result and discussion:

Antimicrobial activity was successfully observed and results were expressed as zone of inhibition. The diameter of zone of inhibition was measured. The results were as follows,

By disc method

From the above result of Disc method, we conclude that the different extract (Sandal wood and Turmeric) show the anti-microbial activity against the isolated organism from normal flora of skin but for the prevent contamination in the result we will choice three isolated out of seven organism (Table 1).

By well method

In the well method we were show the anti microbial activity of extract (Turmeric and Sandal wood) against normal flora of skin. It shows that the A_4 organism was more susceptible as compare to A_3 and F_1(Table 2-4).

By turbidometer method

The MIC of the Sandal wood and Turmeric extract for different organism isolated from normal flora of skin(Table 5-6).

1. In A_3 organism the MIC of Sandal wood was 0.125 and Turmeric was 0.389 at the concentration 40 µl.
2. In A_4 organism the MIC of Sandal wood was 0.011 and for Turmeric was 0.760 at the concentration 40 µl.
3. In F_1 organism the MIC of Sandal wood was 0.342 and for Turmeric 0.270 at the concentration 40 µl.

Thus the medicinal plant Santalum showed anti-bacterial activity in regards to the test organisms. Comparative studies were made as follows:

- The test organisms A_3, A_4 and F_1 were inhibited by the ethanol-extract of the plant.
- Highest degree of inhibition was seen against A_4 with ethanol-extract of Santalum and Curcuma as compare to A_3 and F_1.
- Ethanol-extract of the plant showed anti-bacterial activity against the isolated organisms.
- A_3 was inhibited by ethanol-extract of Santalum with zone of inhibition was 2.13cm(40 µl), 2.3cm (60 µl), 2.43cm (80 µl), 2.8cm (100 µl), 2.5cm(120 µl) and for Turmeric with zone of inhibition was 1.96cms(40 µl),2.46cm (60µl),2.76cm(80µl),3.0cm(100µl), 3.06cm(120µl) respectively.
- F_1 was inhibited by ethanol-extract of Santalum with zone of inhibition was 1.23cm(40 µl), 1.7cm (140 µl), respectively.

Table 1: Antimicrobial Activity of ethanol-extract of Santalum album and curcuma longa against A_3 organism.

Concentrations	Zone of inhibition	
	Sandal wood	Turmeric
A_3	1.3 cm	0.76 cm
A_4	0.56 cm	0.96 cm
F_1	0.86 cm	0.96 cm
F_2	0.93 cm	0.80 cm
F_3	0.7 cm	No zone of MIC

Table 2: Antimicrobial Activity of ethanol-extract of Santalum album and curcuma longa against A_3 organism.

Concentrations	Zone of inhibition	
	Sandal wood	Turmeric
40 µl	1.33 cm	1.93 cm
60 µl	1.7 cm	1.9 cm
80 µl	1.93 cm	2.3 cm
100 µl	2.03 cm	2.4 cm
120 µl	2.1 cm	2.8 cm

Table 3: Antimicrobial Activity of ethanol-extract of Santalum album and Curcuma longa against A_4 organism.

Concentrations	Zone of inhibition	
	Sandal wood	Turmeric
40 µl	2.13 cm	1.96 cm
60 µl	2.3 cm	2.46 cm
80 µl	2.43 cm	2.76 cm
100 µl	2.8 cm	3.0 cm
120 µl	2.5 cm	3.06 cm

Table 4: Antimicrobial Activity of ethanol-extract of Santalumalbum and Curcuma longa against F_1 organism.

Concentrations	Zone of inhibition	
	Sandal wood	Turmeric
40 µl	1.23 cm	1.33 cm
60 µl	1.93 cm	2.13 cm
80 µl	1.86 cm	2.03 cm
100 µl	2.3 cm	2.36 cm
120 µl	1.4 cm	2.26 cm

Table 5: For Sandal Wood

Concentration	Organism		
	A_3	A_4	F_1
10 µl	0.550	0.077	0.413
20 µl	0.182	0.041	0.364
30 µl	0.141	0.031	0.344
40 µl	0.125	0.010	0.342

Table 6: For Turmeric

Concentration	Organism		
	A_3	A_4	F_1
10 µl	0.536	0.270	0.410
20 µl	0.430	0.092	0.387
30 µl	0.424	0.038	0.304
40 µl	0.389	0.760	0.270

Summary and Conclusion:

The medicinal plant *Santalum album and Curcuma longa* showed antibacterial activity in regards to the normal skin flora organisms. The ethanol-extract showed varying degrees of antimicrobial activity on the microorganisms tested. The chance to find antimicrobial activity was more apparent in ethanol extracts of the medicinal plant. Three organism were inhibited by the ethanol-extract of the plant. Some organisms are more susceptible on both extract and some are less. This possibly means that the compound responsible for antimicrobial activity was present in the extract at a different concentration.

This *in vitro* study demonstrated that secondary metabolites extracted from Santalum and Curcuma leaf-extracts can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of this plant suggests that they represent an economic and safe alternative to treat infectious diseases. However, water-extract of the plant is not recommended in the treatment of infections produced by *E.coli* and *S.aureus*because the compounds (secondary metabolites) responsible for antimicrobial activity are not extracted.

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