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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CHROZOPHORA TINCTORIA* AND *ARISTOLOCHIA BRACTEOLATA* YEMENI PLANTS USED IN FOLK MEDICINE.

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Abstract

The research on medicinal plants is gradually gaining popularity due to millions of people depending on the use of different parts of these materials for various ailments.

The present study aims to screen and study two selected Yemeni medicinal plants *Chrozophora tinctoria L.* and *Aristolochia bracteolata L.* for their phytochemical constituents and antibacterial activity against two resistant bacterial pathogens Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* using the disc diffusion method. The solvents used for the extraction of plant leaves, stem and fruits were 90% methanol and absolute methanol.

The phytochemical constituents of various parts of these plants were investigated using standard methods of phytochemical screening. Qualitative screening revealed that tannins, phenols, flavonoids glycosides and anthroquinones were present in all the parts of the plants, whereas triterpinoids, steroids, volatile oils and cardiac glycosides were absent in all parts of the *C. tinctoria.* On the other hand, saponins were absent in all the parts of the *A. bracteolata*, while alkaloids, saponins and flavonoids were present only in some parts of the *C. tinctoria*.

The antibacterial activity revealed that the leaves and stem 90% methanolic extract of *C. tinctoria* gave the widest zone of inhibition against the *Staph. aureus* but no inhibition was observed against *Ps. aeruginosa*, while methanol extract of *A. bracteolata* did not show any activity against *Staph. aureus* and *Ps. aeruginosa*.

Introduction:-

The worldwide use of natural products including medicinal plants has become more and more important in drug discovery and primary health care. The traditional medicine still plays an important role in the primary health care in

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Yemen [1]. Medicinal plants contain natural compounds, which provide definite physiological action on the human body, and these bioactive substances include alkaloids, flavonoids, terpenoids and steroids [2,3].

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [4].

As the microorganisms increasing their resistance to antibiotic hence, the activity of those antibiotics is reduced, and this complicate the problems that medicals and veterinaries are dealing with nowadays leading the researchers to investigate new antibiotics to the resistance strains [5].

***Chrozophora tinctoria* (L.):-**
Is an annual herb commonly known as 'dyers-croton'; 'giradol' and it belong to the family *Euphorbiaceae* [6]. This species distributed in the Mediterranean regions, tropical Africa and West Asia [7]. It is used to treat warts, cathartic, emetic and fever [8]. The leaves are used in chest burning in Kadhi areas of Khushab [9]. Root ashes are given to children for cough, while its bark is used for tanning and dyeing [10]. Several biological activities like antioxidant, antimicrobial and cytotoxic effects have been reported from plant extracts [11,12]. Many phytochemical constituents have been reported from *Chrozophora tinctoria* including flavonoids, flavonoid glycosides, phenylpropanoid glycosides and dolabellanediterpene glucosides [13-15].

Furthermore, twelve compounds were isolated from *Chrozophora tinctoria*. They were identified as quercetin, quercetin 3-*O*-β-glucopyranoside, quercetin 3-*O*-(6"-α- rhamnopyranosyl)-β glucopyranoside, kaempferol, kaempferol 3-*O*-β-glucopyranoside, kaempferol 3-O-(6"-arhamnopyranosyl)-β-glucopyranoside, apigenin, apigenin 7-*O*-bglucopyranoside, acacetin, gallic acid, methyl gallate and β-sitosterol-3-*O*-β-glucopyranoside [16]

***Aristolochia bracteolata* Lam:-**
Is shrub distributed throughout southern regions of Yemen and tropical region. It belongs to the family *Aristolochiaceae* and commonly called as Worm killer in English and 'Wullaiya, Zarawand, Luwayaiya' in Yemen [17]. In folk medicine *A. bracteolata* is used as a gastric stimulant, purgative and in the treatment of cancer, dysentery, lung inflammation, skin diseases, eczema, and snakebites. Seeds used for softening hair [18-20]. *A. bracteolata* has been reported for hypothermia, hypotensive, anti-inflammatory, antibacterial, antifungal and antioxidant properties [20,21]. It also possess a potent anti-allergic activity [22]. The phytochemical investigation of Indian *A. bracteolata* has revealed the presence of alkaloids, triterpenoids, steroids, sterols, flavonoids, phenolic compounds and cardio glycosides [23,24]. Aristolochic Acid-I and Aristolochic Acid-II were isolated and identified from whole of this species [25].

In the present work, qualitative phytochemistry analysis and antibacterial activity were carried out in two wild plants, *Aristolochia bracteolata* and *Chrozophora tinctoria* of south region of Yemen.

Materials and Methods:-

Plant collection:-
The fresh and healthy plant parts of *A. bracteolata* and *C. tinctoria* leaves, stem and root were collected from Al-Mekhlaf, Taiz, Yemen, in the summer (July 2015). The plants material were cleaned, chopped into small pieces and dried under shade for three to four weeks. They were then ground into powder. The identity of the plants were confirmed by Dr. Hassan M. Ibrahim (Taxonomist), Biology Department, Faculty of Science, Sana'a University, Yemen.

Preparation of plant extract:-
The dried plants materials (160 g) were extracted by direct maceration at room temperature until exhaustion with aqueous methanol (methanol/water 90/10) for *Chrozophora tinctoria* and with absolute methanol for *Aristolochia bracteolata* (3 X 1000 ml). The extracts were filtered and evaporated to dryness under low pressure at 40◦C, using rotary evaporator. The different extracts were stored at 4◦C until tested.

Chemicals and reagents:-
Chemicals and reagent used in this study were purchased from Sigma Company, USA and BDH, India and were of highest technical grades.

Phytochemical Screening:-
Qualitative Phytochemical screening of the plant material were performed using a standard procedure [26,27]. The phytochemical analysis of plant samples was carried out as follows:

Test for Alkaloids:-
Alkaloids salts:-
The aqueous extract of each organs of the plant (20 mL) was stirred with 10 mL of 2 % HCl on a steam bath for 30 minutes. The mixture was extracted with three times of diethyl ether. 1 mL of the aqueous layer was treated with three drops of Wagner's reagent. Formation of brownish precipitate was regarded as evidence for the presence of alkaloids salts in the extract.

Free Alkaloids:-
15 mL of organic layer (diethyl ether) was evaporated to dryness. The residue was then dissolved in 2 mL of HCl 2 % and treated with three drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract.

Test for Saponins:-
The sample (1 mg) was shaken with 20 ml of distilled water and then heated to boil. Appearance of foam persisting for 10 min indicated presence of saponins.

Test for Phenolic and Tannins:-
The sample (1 mg) was mixed with 30 ml of distilled water and heated in water bath. The mixture was filtered and ferric chloride $FeCl_3$ was added to the filtrate. Brownish green or a blue-black colouration indicated the presence of phenolic and tannins.

Test for Anthraquinones:-
The powdered plant sample (500 mg) was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% ammonium hydroxide (NH_4OH) solution was added to the filtrate. A violet colour was observed in the lower phase. It indicated presence of anthraquinones.

Test for Flavonoids:-
0.5 g of each selected plant extract were added in a test tube and 10 ml of distill water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H_2SO_4. Indication of yellow color shows the presence of flavonoid in each extract.

Test for cardiac glycosides:-
Killer Killian's test –the extract 0.5g was dissolved in distilled water and then filtered. To 3 ml of filtrate 1ml of glacial acetic acid and a drop of ferric chloride and a drop of concentrated sulfuric acid were added. Green blue color to upper layer and reddish brown color at the junction of two layers indicates the presence of cardiac glycosides

Test for volatile oils:-
To 1ml of the extract dilute HCl was mixed with. A white precipitated was formed which indicated the presences of volatile oils.

Test for Steroids and Triterpenoids:-
Liebermann Burchard test - Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Evaluation of antibacterial activity:-
Selected Bacterial species:-
Methanol and aqueous methanol extracts were challenged against two well-known bacterial species; *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative) isolated from clinical samples and identified

by relevant biochemical tests according to standard procedures [28]. These two species are well-studied in terms of antimicrobial resistance and are commonly employed in research for evaluation of antibacterial activity of plant extracts.

Screening for Antibacterial Activity:-
According to manufacturer instructions, Nutrient agar (HiMedia, India) was prepared and used for cultivation and antibacterial activity testing. The challenged species were cultured on Nutrient agar for 24 hours at 36±1°C. The inoculum suspensions for antibacterial activity testing of each bacterial species were prepared in normal saline solution and adjusted to match the turbidity of 0.5 McFarland standard solution, giving approximately 1.5×10^8 CFU/mL. The antibacterial activity was tested by standard disc diffusion method [29,30]. Sterile filter paper discs were saturated by a total of 10µL of the targeted concentration of extracts by alternative spotting of 5µL on both sides of the discs [31]. Impregnated discs were placed aseptically on the surface of inoculated plates and incubated at 36±1°C for 24 hours. Both negative control (discs impregnated with corresponding concentration of the solvent) and positive control Amoxicillin 25 µg (HiMedia, India) were used in every agar plate for *S. aureus* and *P. aeruginosa* respectively). Antibacterial activities were evaluated by measuring the diameters of inhibition zones in millimeter against the test organisms.

Different dilutions of the plant extracts prepared in the order of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12 mg/ml 6.5 mg/ml respectively were prepared in five different test tubes and placed in a test tube rack.

Results and Discussions:-

Phytochemical analysis is of paramount importance in identifying new source of medicinally and industrially valuable compounds having medicinal plants have been chemically investigated [32]. In the present study carried out on *C. tinctoria* and *A. bracteolata* revealed the presence of medicinal active constituents have been screened. The phytochemical active compounds of *C. tinctoria* and *A. bracteolata* were qualitatively analyzed for leaves, stem and fruits and the results are presented in Table **1**.

Aqueous methanolic extracts of *C. tinctoria* showed the presence of different types of secondary metabolites such as tannins, phenol, flavonoids, flavonoids glycosides, anthroquinones, while, triterpinoids, steroids and volatile oils were absent in *C. tinctoria*.

Aynehchi et al. reported no presence of saponins, alkaloids, tannins and flavonoids in the whole *C. tinctoria* which is in agreement with the observations of our study [33]. This report is also in agreement with that of Begum et al., who reported the presence of alkaloids, flavonoids, tannins, anthroquinones, and saponins in the whole plant of *C. tinctoria* [34].

Methanol extracts from *A. bracteolata* showed the presence of triterpinoids, steroids, volatile oils, cardiac glycosides, anthroquinones, phenol, flavonoids, flavonoids glycosides, tannin and alkaloids while, saponins were not present in *A.bracteolata*. Periyasamy et al. reported no presence of tannin and anthroqunones in the leaves *Aristolochia bracteolata*, while it is revealed in this study [35].

Gbadamosi, 2012 reported the presence of saponins in the root of *A. bracteolata* while it is not revealed in this study but this work revealed the presence of triterpenoids, tannin and anthroquinones in the leaves, fruits and stem bark extract [36].

Table 1:- Phytochemical analysis of extracts from leaves, stem and fruits of *Chrozophora tinctoria* and *Aristolochia bracteolata*

Phytochemical constituents	*Chrozophora tinctoria*			*Aristolochia bracteolata*		
	Leaves	**Stem**	**Fruits**	**Leaves**	**Stem**	**Fruits**
Triterpinoids	-ve	-ve	-ve	+ve	+ve	+ve
Steroids	-ve	-ve	-ve	+ve	+ve	+ve
Volatile oils	-ve	-ve	-ve	+ve	-ve	+ve
Anthroquinones	+ve	+ve	+ve	+ve	+ve	+ve
Cardiac glycosides	-ve	-ve	-ve	+ve	+ve	+ve
Free alkaloids	+ve	-ve	-ve	+ve	+ve	+ve
Alkaloids salts	+ve	-ve	-ve	+ve	+ve	+ve

Saponins	+ve	+ve	-ve	-ve	-ve	-ve
Flavonoids	+ve	+ve	-ve	+ve	+ve	+ve
Flavonoids glycosides	+ve	+ve	+ve	+ve	+ve	+ve
Phenolics/ Tannins	+ve	+ve	+ve	+ve	+ve	+ve

Key: -ve : absence; +ve: presence

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases [37]. For example, flavonoids exhibit a wide range of biological activities which include antimicrobial, anti-allergic effects, anti-angionic, anti-inflammatory, analgesic, cytostatic and antioxidant properties [38].

Saponins have been reported to possess antibacterial property with their mode of action attributed to their ability to cause leakage of proteins and certain enzymes from bacterial cells [39]. In addition, Saponins are known to produce inhibitory effects on inflammation [40]

Tannins have been found to have anti-inflammatory, antioxidant, antiviral, antibacterial, antiulcer and antiparasitic effects, property for possible therapeutic applications. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic [41]

Alkaloids, which are one of the largest groups of phytochemicals in plants, have amazing effect on humans and this has led to the development of powerful painkiller medicines. In addition, alkaloids possess antiinflammatory, antiasthmatic, and antianaphylatic properties with consequences of altered immunological status *in vivo* [42].
Plant steroids are known to be important for their cardiotonic activities and possess antimicrobial and insecticidal properties. They are also used in nutrition, herbal medicine and cosmetics [43].

The results of the antibacterial screening showed that the aqueous methanol of leaves and stem of *C. tinctoria* have potential antibacterial effects against *Staph. aureus*.

It was observed that the leaves extracts gave more antibacterial activity than the other tested part of the plant. This report agrees with that of Dastagir et. al., who reported that the aqueous methanol (methanol/water 70/30 v/v) extracts of Indian *C. tinctoria* had effect against *Staphy. aureus* with 25 mm inhibition zone [10]. On the other hand, the aqueous methanol extracts from leaves and stem of *C. tinctoria* did not show any activity against *Ps. aeruginosa*. Antibacterial activity of methanol extracts of *A. bracteolata* is shown in table **2**. The methanol extract of *A. bracteolata* did not show any activity against *Staph. aureus* and *Ps. aeruginosa*. The present findings seem to be consistent with other research which found that the acetone extract of *A. bracteolata* showed higher antibacterial activity against *Staph. aureus* (15 mm inhibition zone), while, the methanol extract of these species did not show any activity against *Staph. aureus* and *Ps. aeruginosa* [44] **.**

Table 2:- Inhibition Zone diameter of leaves and stem bark of *C. tinctoria and A. bracteolata* extracts

Plant species	**Plant Extracts**	**Conc. of extracts (mg/ml)**	**Zone inhibition (mm) Bacteria strains**	
			S. aureus	**P. aeruginoa**
C. tinctoria	Leaves	100.0	13.0	N.I
		50.0	10.0	N.I
		25.0	8.0	N.I
		12.5	N.I	N.I
		6.5	N.I	N.I
	Stem bark	100.0	8.00	N.I
		50.0	6.25	N.I
		25.0	N.I	N.I
		12.5	N.I	N.I
		6.5	N.I	N.I
A.bracteata	Leaves	100.0	N.I	N.I
		50.0	N.I	N.I
		25.0	N.I	N.I
		12.5	N.I	N.I

		6.5	N.I	N.I
	Stem bark	100.0	N.I	N.I
		50.0	N.I	N.I
		25.0	N.I	N.I
		12.5	N.I	N.I
		6.5	N.I	N.I
Amoxicillin		25 µg	9.0	N.I

Zones of inhibition (mm) for the plant *C. tinctoria* and *A. bracteolata* against the two bacteria under investigation. Positive control was amoxicillin and negative control was methanol. All methanol controls had 0 mm zones of inhibition. N.I = No Inhibition

Conclusion:-

The two native medicinal plants *Chrozophora tinctoria* and *Aristolochia bracteolata* are rich in secondary metabolites like alkaloids, anthroquinones, tannins, flavonoids, steroids, cardiac glycosides etc. presence of various bioactive compounds justifies the use of whole plants for various diseases by traditional practitioners in Yemen.

Relying upon the results obtained from this study it may be concluded that the aqueous methanol extract of *C. tinctoria* showed good antibacterial activity against *Staph. aureus*, whereas the other extracts showed the poor activity. The aqueous methanol used for extraction of medicinal plants is an important factor to obtain optimum antibacterial activity. More detailed study must be done for farther isolation leading to the pure compounds

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