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Chemical constituents study of Umm glagil (*Aristolochia bracteolata*) and antibacterial activity against *Salmonella typhi*

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Abstract

(Aristolochia bracteolata) is widespread used herb in African countries including Sudan. It is as one of the most effective plant for remedies of infectious diseases. The aim of the present study is to investigate the secondary metabolites of Umm glagil (*Aristolochia bracteolata*) in the root and different parts of shoot system such as leave , stems and bark, as well as to study of biological activity as antibacterial against important pathogenic bacteria (*Salmonella typhi*). Different solvents system and aqueous extracts with different concentration (50g/500/ml) and (0.00, 25.0, 50.0, 75.0 and 100 mg/ml) were used. Qualitative analysis were carried out to investigate phytochemical constituents of different parts then after, disc diffusion method (inhibition zone), was used to determinate the sensitivity of the bacteria to the extracts. The phytochemical analysis showed that the extracts of Umm glagil leaf and stem contained flavonoids at moderate concentration, while, flavonones/ flavonols and saponins recorded high concentration in stem. The Methanolic, ethyl acetate, ethanolic and petroleum ether extracts of Umm glagil were more effective against the bacteria than the other solvents giving (1.9, 1.7, 1.3, 1.2 cm) diameters, in order. This study indicated that Umm glagil can be used as antibacterial agents.

Keywords: *Aristolochiabracteolata, Salmonella typhi , antibacterial , Umm glagil , phytochemical.*

دراسة للمكونات الكيميائية لنبات ام جلاجل وفعاليتها المضادة لباكتيريا السالمونيلا

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المستخلص

يُستخدم نبات أم جلاجل (Aristolochia bracteolata) على نطاق واسع في الدول الأفريقية، بما فيها السودان، كأحد أكثر النباتات فعالية في علاج الأمراض المعدية. تهدف هذه الدراسة إلى دراسة المستقلبات الثانوية لأجزاء نبات أم جلاجل (Aristolochia bracteolata) مثل الأوراق والسيقان واللحاء، بالإضافة إلى دراسة نشاطه البيولوجي كمضاد للبكتيريا مثل بكتيريا السالمونيلا التيفية. استُخدمت مذيبات ومستخلصات مائية مختلفة (50 غ/500 مل) ثم بتركيزات مختلفة (0.00، 25.0، 50.0، 75.0 و100 ملغ/مل). أُجري تحليل نوعي لدراسة المكونات الكيميائية النباتية لأجزاء النبات المختلفة، ثم استُخدمت طريقة الانتشار القرصي (منطقة التثبيط) لتحديد حساسية البكتيريا للمستخلصات. أظهر التحليل الكيميائي النباتي احتواء مستخلصات أوراق وساق أم جلاجل على فلافونويدات بتركيزات متوسطة، وفلافونونات/فلافونولات، وصابونينات بتركيزات عالية في الساق. وأظهرت منطقة التثبيط أن مستخلصات الميثانول، وأسيتات الإيثيل، والإيثانول، وإيثر البترول من أم جلاجل كانت أكثر فعالية ضد البكتيريا من المذيبات الأخرى، حيث أعطت أقطارًا (1.9، 1.7، 1.3، 1.2 سم) على التوالي. كما أظهرت الدراسة أن أوراق وساق أم جلاجل أكثر نشاطًا من الساق. وأشارت هذه الدراسة إلى إمكانية استخدام أم جلاجل كمضادات للبكتيريا

الكلمات المفتاحية : *نبات ام جلاجل ، باكتريا السانمنيلا ، مضاد باكتيري ، كيماويات نباتيه.*

Introduction

Medicinal plants for thousands of years played a vital role in human life (Abdel Karim *et al*., 2022). Medicinal plants have provided the basic building blocks for a number of highly effective drugs (Alluri and Majumdar, 2014). They are used for discovering and screening the phytochemical constituents which are very much helpful for the manufacturing of new drugs (Tiwari *et al*., 2011). Phytochemicals are primary and secondary compounds and they are found as major constituents of some medicinal plants which are useful for healing as well as for curing human diseases (Nostro *et al*., 2000). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). However, *Aristolochia* is an important genus in the family of Aristolochiaceae.

The Aristolochiaceae Family consists of about 400 species. It is widespread through tropical areas of Asia, Africa, and South America (MacMillan, 2008) .*Aristolochia* species has been diverse biological functions include hypertension relief, rheumatism relief, edema therapy, leukocyte enhancement, as well as analgesic and diuretic effects (Tang and Eisenbrand, 1992).

Salmonella sp are found in the intestinal tract of wild and domesticated animals and humans. Some serotypes of *Salmonella*, such as *S. typhi* and *S.paratyphi* are only found in humans (Miller and Pegues, 2005). Some serotypes of *Salmonella* have become resistant to many antimicrobial drugs. The selection of effective antibiotics is critical for the treatment of invasive *Salmonella* infections, but has become more difficult as antibiotic resistance has increased, (Consumer reports, 2010).

Objective: To study the chemical constituents of Umm glagil (*Aristolochia bracteolata*) and its antibacterial activity against *Salmonella typhi*

Materials and methods:

Plant collection:

Samples of the plant of Umm glagil (*Aristolochia bracteolata*)leaves, Stems and root were collected from Wad Medani area .Sudan .

Phytochemical analysis:

General phytochemical examination of secondary metabolites (Qualitative analysis) were included the following tests:

Flavonoids : Five gram (5gm) of the dried powder of each part of the plant, was macerated in 1% of hydrochloric acid (50 ml) over night, filtered and the filtrate was subjected to the following tests:

a) Ten ml (10 ml) from each filtrate was rendered alkaline with sodium hydroxide (10%, w/v); if a yellow colour was formed that might indicate the presence of flavonoids.
b) Shinoda test: Five ml (5 ml) of each filtrate was mixed with concentrated hydrochloric acid (1ml) and magnesium turning was added. The formation of red colour indicates the presence of flavonoids, flavonones, and / or flavonols (Harborne. 1998).

Saponins: A known weight (5 gm) of the dried powder of sample was extracted with 20 ml ethanol (50%) and filtered. Aliquots of the alcoholic extracts (10 ml each) were evaporated to dryness under reduced pressure. The residue was dissolved in distilled water (4ml) and filtered. The filtrate was then vigorously shaken; if a voluminous forth is developed and persisted for almost one hour, it this an indication for the presence of saponins (Harborne. 1998).

Tannins: The dried powder of plant sample (5 gm), were extracted with ethanol (50%) and filtered. Ferric chloride reagent (5%, w/v in methanol) was added. The appearance of green color which changes to a bluish black color or precipitate, indicates the presence of tannins (Balbaa, 1974)

Sterols and /or triterpenes: The dried powder of plant sample (1 gm), were extracted with petroleum ether (10 ml each) and filtered. The filtrate was evaporated to dryness and the residue was dissolved in chloroform (10 ml). aliquots of chloroform extract (3 ml each) were mixed with concentrated acetic anhydride (3 ml), and a few drops of sulphuric acid were added. The formation of a reddish violet ring at the junction of the two layers, indicates the presence of unsaturated sterols and / or triterpenes (Harborne. 1998).

Alkaloids and / or nitrogenous bases: The dried powder of each parts plant (5 gm), were extracted with ethanol and filtered. Aliquots from the ethanolic extract (10 ml each) were mixed with aqueous hydrochloric acid (20 ml 10% v/v), and filtered. The filtrate was rendered alkaline with ammonium hydroxide and extracted with successive portions of chloroform. The combined chloroform extract was evaporated to dryness, the residue was dissolved in hydrochloric acid (2 ml 10% v/v) and tested with Mayers reagent, and Dragendorffs reagent, respectively. If a precipitate was formed, it indicates the presence of alkaloids and /or nitrogenous bases (Balbaa, 1974).

Microorganism's sources

The cultures of bacteria (*Salmonella* sp.) obtained from the medical laboratory, University of Gezira, Sudan.

Preparation of medium :

Nutrient agar (N.A) : Twenty-eight grams of (OXOID Ltd.), were dissolved in one litter of distilled water, and then dispensed into flasks (250 ml), and autoclaved at 121°C (15 lb/in^2) for 15 minutes, then poured into sterile Petri dishes, which were allowed to solidity and kept inverted position in a refrigerator before use.

Effect of plant extracts on bacterial growth:

Antibacterial activity was determined by the disc diffusion method (Rıos and Recio, 2005). Where standardized bacterial cell suspensions of *Salmonella typhi* were added to the solidified medium into the sterile Petri dishes. The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured. According to this technique, micro-glass fiber discs of 0.5 in diameter were saturated with 20 µl/ disc crude extract, and placed on the middle of Petri-plates separately. The test organism was streaked on each test Petri-plate prior to the placement of the saturated disc on mid-plate. Treated plates (three replicas for each test) were incubated at room temperature. Zones of inhibition were determined at 24 h post-treatment.

Results and Discussion:

Phytochemicals are naturally occurring compounds that have defense mechanism and protect plants from various diseases. The plant parts containing some phytochemical components in different concentration. Result in Table (1) displayed that the plant'sleaf has high saponine concentration while in low concentration in stem, also it contain flavonoids in a moderate level in other tested parts and contain Flavonones/ flavonols in low concentration .The results were in agreement with that reported by Encarnacion *et al*.(1994). However, the results were differ to that obtained by (Thirumal et al, 2012), who reported that this plant contain alkaloids, tri-terpenoids, steroids, tannins, phenolic compounds and cardiac glycosides.

Table (1): Qualitative chemical screening of Umm glagil plant parts:

The symbol +++, ++,+,- indicate a compound present in high, moderate, low, and absent level, respectively

Plant species	Plant part	Flavonoids	Flavonones / flavonols	Saponins	Tannins	Sterols	Alkaloids
Umm glagil (*Aristolochia bacteolate*)	Leaf	++	+	+++	-	-	-
	Stem	++	+	+	-	-	-

The plant materials of *Aristolochia bracteolata* selected for the study to evaluate the formulation containing the extracts of these plants for their antimicrobial activity. Data in Tables (2 – 5) showed that the effect of the solvent extracts on the *S. typhi* by measuring the inhibition zone diameter(mm). From the results it is highly noticeable that all the tested parts of Umm glagil methanolic extracts have

reasonable effect against *S. typhi*. followed by ethyl acetate of Umm glagil leaf and stem at the high concentration (100%). On the other hand, methanolic extracts root were also show good effect against *S. typhi*. The presence of secondary metabolites in plants, produce some biological activity. *Aristolochia* is used in traditional medicine for the treatment of various diseases, including those associated with bacteria. Phenols and tannins known us possess antibacterial properties (Palombo, 2011). According to (Sampedro and Valdivia, 2014), alkaloids, phenylpropanoids, or flavonoids, and terpenoids, which include saponins, are significant plant antibacterial agents. El Dirdiri *et al*., (1987) reported that root extract have antibacterial activity

A positive control was conducted by using antibiotic disks for Gram positive and Gram negative bacteria. The test was done to compare the effect of these antibiotic with the plant extract under the present study.

Table (2) : Effect of Umm glagil plant parts extracts obtained by different solvents on *S . typhi.*

Solvents	Plant parts		
	Leaf (mm)	**Steam(mm)**	**Root(mm)**
Ethyl acetate	17	15	05
Ethanol	13	09	05
Hexane	05	05	05
Methanol	18	19	17
P. ether	09	12	05
Water c	05	05	05

Table (3) : Effect of different concentrations of *Umm glagel* Ethayl acetae extracts on *S. typhi* .

Concentration %	Plant parts		
	Leaf(mm)	Steam(mm)	Root(mm)
0	05	05	05
25	10	05	05
50	14	11	05
75	16	12	05
100	17	15	05

Table (4) : Effect of different concentrations of *Umm glagel* Methanol extracts on*S. typhi*

Concentration %	Plant parts		
	Leaf	Steam	Root
0	05	05	05
25	13	14	12
50	15	15	13
75	17	17	15
100	18	19	17

Table (5) : Effect of different concentrations of *Umm glagegl* P.ethar extracts on*S. typhi*

Concentration %	Plant parts		
	Leaf (mm)	Steam(mm)	Root(mm)
0	05	05	05
25	05	07	05
50	06	08	05
75	08	10	05
100	09	12	05

Using gram negative antibiotic disk, the test was done to compare the effect of these antibiotic with the plant extract under study. The results are presented on table (6) which showed that the plant extract have an almost similar effect of inhibition as a standard antibiotics Piperacillin/ Tazobactam (TZP),Chloramphenicol (CH) and Tetracycline (TE). The plant extract recorded better effect than Co-Trimoxazole (BA) , Ciprofloxacin (CP) and Ceftizoxime (CL).

Table(6) : Effect of Gram negative antibiotic (on inhbition zone-cm) against *S. typhi*

Antibiotic	*S.typhi*
Ampicillin/ Sulbactam (AS)	2.0
Co- Trimoxazole (BA)	1.0
Cefotaxime (CF)	0.0
Piperacillin/ Tazobactam (TZP)	1.7
Chloramphenicol (CH)	1.8
Ciprofloxacin (CP)	1.0
Ceftizoxime (CL)	1.0
Tetracycline (TE)	1.2
Ofloxacin (OF)	2.5
Gentamicin (GM)	3.00
Amikacin (AK)	2.0
Levofloxacin (LE)	2.3

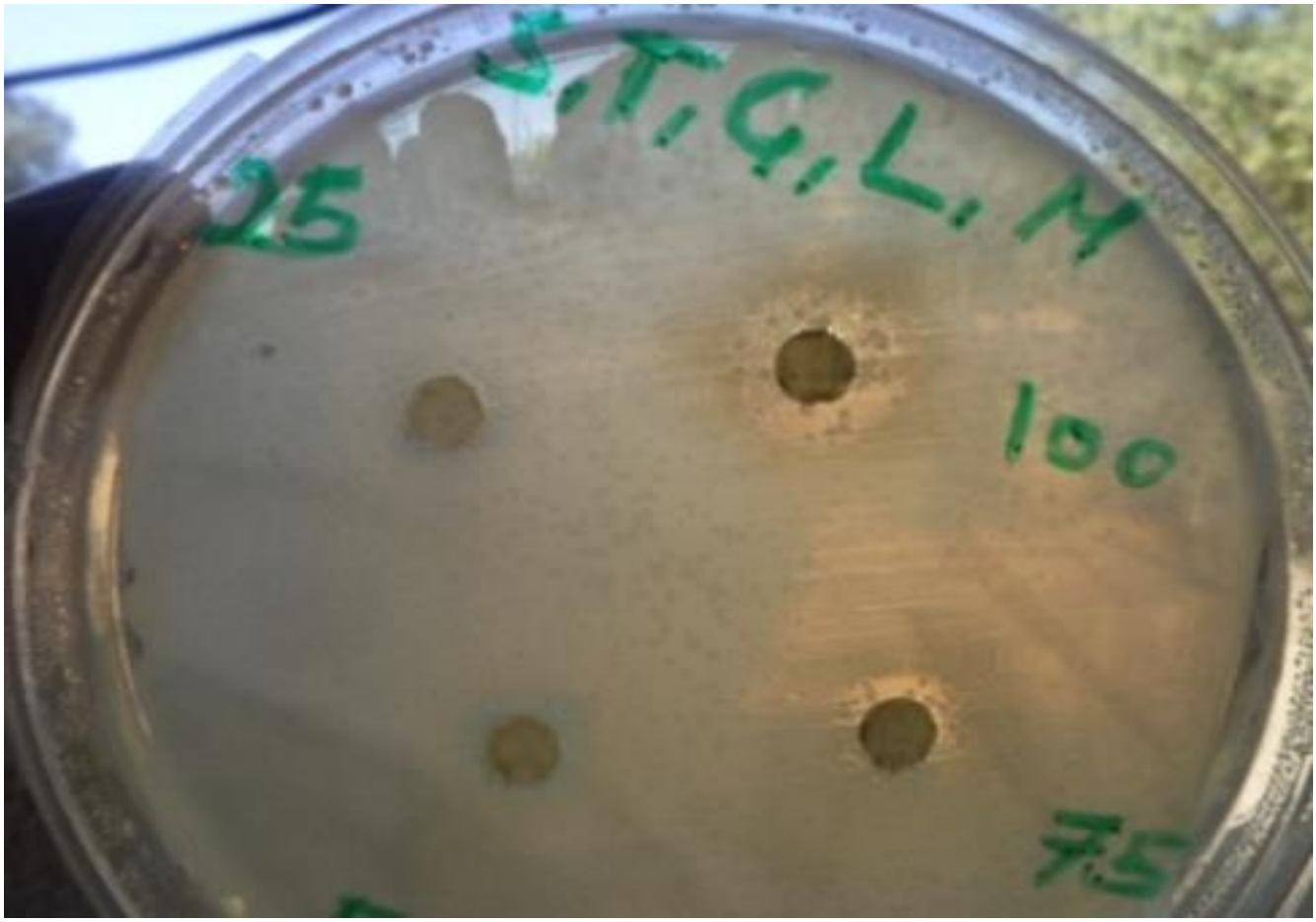

Figure (1) : photograph of umm glagil methanolic leaf extract against *S. typhi*

Conclusion

It could be concluded that , *Aristolochia bracteolate* regarding the present study this plant has active phytochemical compounds as antibacterial activity of all tested parts to mainly against *salmonella typhi.*

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