GC-MS analysis, antioxidant and antibacterial activity of acetone fractions obtained from *Guiera Senegalensis* leaves and *Quercus Infectoria* Nutgalls extracts

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Abstract

Medicinal plants are considered an important source of phytochemical compounds that play a vital role to produce a definite physiological action for the treatment of several diseases. This study was aimed to investigate the phytoconstituents of *Guiera senegalensis* leaves and *Quercus infectoria* nutgalls extracts, and to study their antioxidant and antibacterial activities. Ethanol extracts were subjected to sequential fractionation using petroleum ether, dichloromethane and acetone. Acetone fraction (as major) was analyzed using qualitative and quantitative GC-MS analysis. *In vitro* antioxidant activity and antibacterial sensitivity against *Staphylococcus aureus* of acetone fraction were evaluated whereas; DPPH radical scavenging activity and disc diffusion methods were used, respectively. Results obtained from GC-MS analysis for *G. senegalensis* were showed the presence of eupafolin, pyrogallol, hydroquinone and catechol with percentages of 65.16%, 15.79%, 10.36% and 8.69%, respectively. While *Q. infectoria* GC-MS analysis was revealed the presence of pyrogallol as major phytoconstituent (94.77%). The antioxidant activity of the two extracts showed higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (94%) at a concentration of 250 µg/ml compared to standard popylgallate. Both extracts at a concentration of 25 mg/ml were exhibited higher antibacterial activity against *S. aureus* compared to gentamicin. It could be concluded that *G. senegalensis* leaves and *Q. infectoria* nutgalls possess significant antioxidant and antibacterial principles for possible treatment of inflammations and bacterial infections especially those caused by *S. aureus*. Further experimental and clinical studies are warranted.

Keywords: *Guiera senegalensis, Quercus infectoria,* Acetone fraction, GC-MS,Antioxidant, ntibacterial.

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Introduction

Plants are used for discovering and screenings of phytoconstituents to provide new chemical entities that useful in manufacturing of novel drugs to control various diseases (Katiyar *et al.* 212; Lautié *et al.* 2020). Knowledge of their medicinal properties is growing as a result of research and testing, which will make them an increasingly safe alternative or a preferred option to allopathic medicine (Ekor 2014). Today, there is a re-invite attention in folkloric medicine and an increasing request for more drugs from plant sources (WHO 2019). This revival of interest in plant derived medicines is mainly due to the current pervasive belief that "green medicine" is safe and more credible than the costly synthetic medicines, many of which have adverse reaction (Parekh and Chanda 2006). Oxidation is necessary for energy production in all living systems; it can produce free radicals which can start chain reactions that may damage cells. Antioxidants of plant origin such as thiols and polyphenols act as reducing agents and terminate these chain reactions by removing radical intermediates (John *et al.* 2012). Phenolic compounds are important in the defense mechanisms of plants under different environmental stress conditions such as wounding and infections (Heleno *et al.* 2015). *Staphylococcus aureus* is one of the most common bacterial pathogen that causing skin and wound infections (Ayub *et al.* 2015). Now microorganisms have become resistant to many antibiotics due to increase their misuse. Recently, medicinal plants with their different phytoconsistituents have gained importance as potential antibacterial agents which may contribute to find out new antibacterial and anticancer agents (Lautié *et al.* 2020). *G. senegalensis* (Combretaceae) and *Q. infectoria* (Fagaceae) commonly known as Ghubaysh and Affsa are small trees or shrubs widely distributed in the savannah region of West and Central Africa and Western Sudan. They are known plants in Sudan folkloric medicines used as dental powder for toothache, gingivitis, and for the treatment of inflammatory diseases (Jain *et al.* 2019). Their extracts have been applied topically in the treatment of wounds, injuries, psoriasis, eczema, and different skin disorders (Kaur *et al.* 2004; Aniagu *et al.* 2005; Al Shafei *et al.* 2016). The most phytoconstituents reported in *G. senegalensis* and *Q. infectoria* are including galloylquinic acid derivatives, simple phenolic compounds and flavonoids (Tayel *et al.* 2018; Dirar and

Devkota 2021). This study aimed to identify the major phytoconstituents pertaining to the medicinal activity of the plants using GC-MS technique, as well as to confirm the antioxidant and antibacterial activities of the acetone fractions of the two plant extracts.

Materials and methods

Plant materials

Fresh leaves of *G. senegalensis* and nutgalls of *Q. infectoria* were collected from Ghubaysh area of Western Sudan. The plant materials were identified and authenticated by taxonomist at the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for research (NCR), Sudan.

Extraction of plant materials

The plant materials were separately washed with sterile distilled water to remove dirt and impurities and dried in shadow air, crushed in a mortar into a coarse powder. About 500g of coarsely ground plant material was extracted by maceration using 80% ethanol for 72 hours .Each of the obtained ethanolic extract was sequentially fractionated with petroleum ether for defatting, dichloromethane and acetone by soxhelt extractor (Sarika *et al.* 2018). After filtration through Whatman filter paper No. 1 each of the solvents was evaporated under reduced pressure using a rotary evaporator at 40˚C to obtain the concentrated fraction, transferred to small container and stored in a refrigerator at 4˚C until tested.

GC-MS analysis of acetone fractions

The acetone fractions of *G. senegalensis* leaves and *Q. infectoria* nutgalls were analyzed separately for their chemical composition using GC-MS systems. The GC-MS analysis was performed on Simadzu (GC\MS QP2010-Ultra, Helium was used as carrier gas and the separation was achieved using a Restek fused silica capillary column (Rxi-5ms:30m×0.25 mm×0.25µm i.d, 0.25 µm film thickness). The starting oven temperature was programmed at 50˚C with increasing temperature of 7˚C until reached 180˚C, then the rate was changed to 10 c/min reaching 300c as final temperature degree. Flow rate 1.69 ml/min, the injection port temperature was 300˚C, the ion source temperature was 200˚C and the interface

temperature was 250˚C. The GC-MS analysis was performed by using scan mode in the range of m/z 40-550 charges to ratio and the total run time was 28 minutes (Sombié *et al.* 2013). The percentage composition of the crude extract constituents was expressed as a percentage by peak area. Identification and characterization of chemical compounds in the plant materials were based on comparing their retention times and mass spectra fragmentation pattern with National Institute of Standards and Technology (NIST) library of GC-MS data system.

Antioxidant activity of acetone fractions

The radicals scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was circumscribed according to the method described by Rahman *et al.* 2015 with some modification. Sample stock solution (1 mg/ml) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in DMSO. One ml of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in ethanol solution was added to a 2.5 ml solution of the different concentrations of the fraction and allowed to react at room temperature for 30 minutes at 37˚C. A freshly prepared DPPH solution exhibits a deep violet colour with an absorption maximum at 517 nm. This violet colour generally fades when antioxidant molecule quench DPPH free radicals (Amarowicz *et al.* 2004). After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with DMSO treated control group. Propyl gallate was used as the antioxidant standard. Tests were carried out in triplicate. Percentage of radical scavenging activity was calculated using the following expression:

$$\% \text{ of inhibition} = ({}^{A}\text{blank} - {}^{A}\text{sample}) / {}^{A}\text{blank} \times 100$$

Where: ^{A}blank and ^{A}sample stands for absorption of the blank and absorption of tested sample extract solution respectively.

In vitro antibacterial activity of acetone fractions against *S. aureus* standard strain

The paper disc diffusion method was used to screen the antibacterial activity of plant extract and performed by using Mueller Hinton agar (MHA) (Jonasson *et al.* 2020). Twenty mg of extract was dissolved in 2 ml of ethanol, serial dilution of different extract concentrations of 100, 50, 25, 12.5and 6.25mg/ml were prepared. Standard *S. aureus* (American Type Culture Collection, ATCC25923, Rockville, Maryland, USA) bacterial suspension was diluted with sterile physiological solution to 108 cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extract. The introjected plates were incubated at 37 °C for 24 h in the inverted position. The standard discs of the antibiotics gentamycin (30 mg per disc) served as positive antibacterial control. Tests were carried out in triplicate. The diameter of zones of inhibition around each of the discs (disc diameter included) was taken as a measure of the antibacterial activity; the mean diameters (mm ± SD) of the zones of inhibition were calculated (Ayub *et al.* 2015; Tong *et al.* 2015; Jonasson *et al.* 2020).

Statistical analysis

The results were analyzed using Statistical Package for Social Science program (SPSS) version 10 and expressed as a mean ± standard deviation (SD). The results were considered statistically significant at $p < 0.05$.

Results and discussion

The yield percentages of ethanolic maceration for powdered plants were 40% and 80 % for *G. senegalensis* and *Q. infectoria*, respectively. Acetone fractions after successive fractionation in both extractive yields were further analyzed. GC-MS analysis was revealed the presence of several compounds including: flavone eupafolin, pyrogallol, hydroquinone and catechol in *G. senegalensis* (Table 1). This result is similar to the findings noticed by Sombié, *et al.* 2013 and Eltoum *et al.* 2020, whom identified the presence of flavonoids by GC-MS in *G. senegalensis* methanol extract.

Table (1): Phytochemical components identified with GC-MS of *G. senegalensis* acetone fraction of leaves alcoholic extract

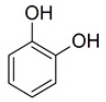
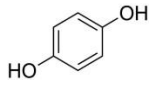
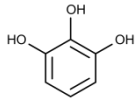
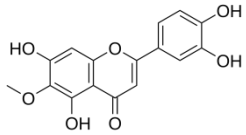
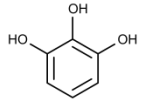
Peak No	IUPAC Name	Common Name	Class	Formula	Structure	Retention Time	Area	Area %
1	1,2-Benzenediol	Catechol	Tannins	$C_6H_6O_2$		11.054	232646	8.69
	Benzene-1,4-diol	Hydroquinone	Phenol	$C_6H_6O_2$		12.210	277129	10.36
3	Benzene-1,2,3-triol	Pyrogallol	Tannins	$C_6H_6O_3$		15.145	690228	15.79
4	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-methoxychromen-4-one	Eupafolin	Flavone	$C_{16}H_{12}O_7$		29.360	146002	65.16

Table (2): Phytochemical component identified with GC-MS of *Q. infectoria* acetone fraction of nutgall alcoholic extract

Peak No	IUPAC Name	Common Name	Class	Formula	Structure	Retentio Time	Area	Area %
1	Benzene-1,2,3-triol	Pyrogallol	Tannins	$C_6H_6O_3$		15.10	97057262	94.77

GC-MS analysis for *Q. infectoria* was indicated that pyrogallol is presents in high concentration (94.77 %) as showed in Table 2. This result is coincides with the study indicated that GC-MS analysis of *Q. infectoria* methanol extract was showed high percentage of pyrogallol (81.66%) of extract (Saeida *et al.* 2014). Also, another study indicated that *Q. infectoria* is containing high concentration of phenolic compounds and flavonoids which obtained by Liquid chromatography and tandem mass spectrometry (LC-MS/MS) analysis (Hamad *et al.* 2017).

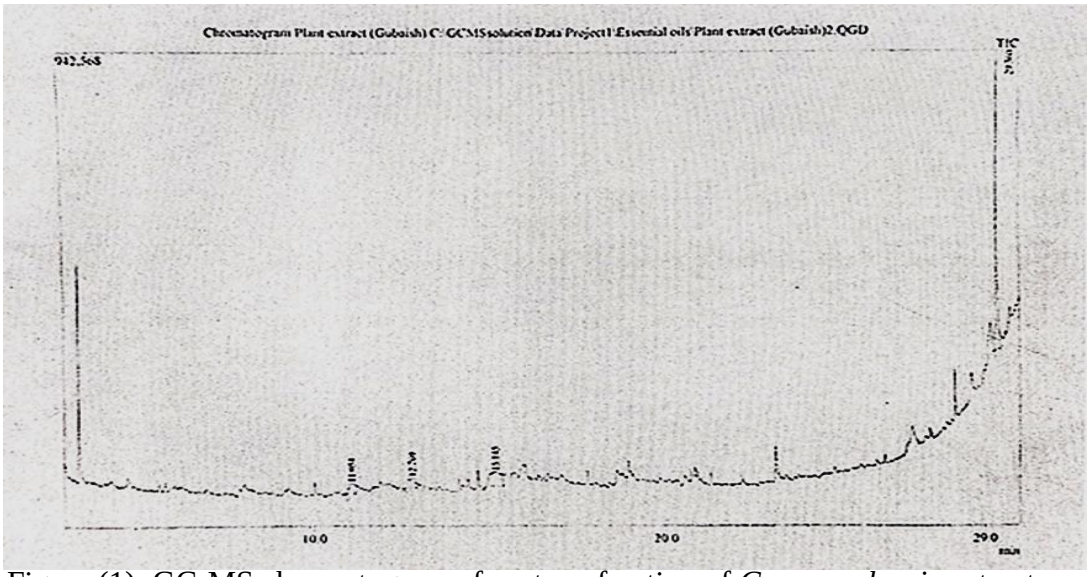


Figure (1): GC-MS chromatogram of acetone fraction of *G. senegalensis* extract

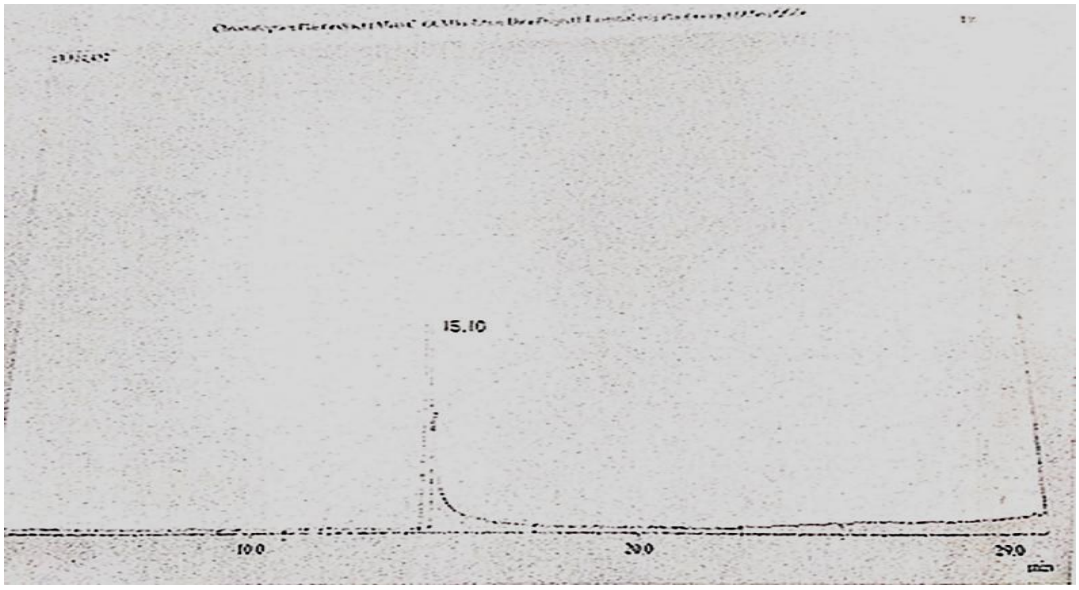


Figure (2): GC-MS chromatogram of acetone fraction of *Q. infectoria* galls extract

Antioxidant activity of the acetone fractions

The antioxidant activity for two fractions was determined using DPPH free radicals scavenging activity compared to standard antioxidant propylgallate and expressed in terms of inhibition (%) as shown in Table (3). At a concentration of 250 µg/ml, both acetone fractions scavenged 94% of DPPH free radicals, while a moderate scavenging activity of 72% was produced at the low concentration as 5µg/ml which was found more than that exhibited by the standard propylgallate 90 % and 55.5 % with concentrations 250 µg/ml and 5µg/ml, respectively. Polyphenolic compounds as flavonoids and tannins are major ubiquitous components of many plant extracts that responsible for the free radical scavenging, anti-inflammatory, analgesic and antimicrobial activities (Süntar *et al.* 2012; Ahlem *et al.* 2015). *G. senegalensis* and *Q. infectoria* antioxidant activity is points to the high content of phenolic components including tannins and flavonoids therein and their biological and therapeutic activities in fighting free radicals and preventing oxidative stress (Shetty *et al*. 2008; Abubakr *et al.* 2013; Nur *et al.* 2014; Sarika *et al.* 2018; Hatim *et al* 2020).

Table (3): Percentage of DPPH radicals scavenging activity of acetone fractions

Sample	**Concentration and DPPH inhibition (%)**				
	250 µg/ml	125 µg/ml	50 µg/ml	10 µg/ml	5µg /ml
G. senegalensis	94 %	91 %	89.7 %	79.5 %	72.5 %
Q. infectoria	94 %	92 %	90.6 %	82.5 %	72.4 %
Propyl gallate	90 %	90 %	72.5 %	62.1 %	55.5 %

Table (4): Antibacterial sensitivity of acetone fractions

Concentrations (mg/ml)	**Mean zone of inhibition (mm ± SD).**		
	Gentamycin	*G. senegalensis*	*Q. infectoria*
30	14 ± 0.01	-	-
100	-	20 ± 0.01	23 ± 0.01
50	-	19 ± 0.01	20 ± 0.01
25	-	18 ± 0.01	18 ± 0.01
12.5	-	15 ± 0.02	18 ± 0.01
6.25	-	15 ± 0.02	16 ± 0.02

*Sensitivity of *S. aureus*: > 18 mm: Sensitive, 14-18 mm: Intermediate, <14 mm: Resistant (Mounyr *et al.* 2016).

In vitro antibacterial activity of acetone fractions

Antibacterial activity of acetone fractions against *S. aureus* is presented in Table 4, which indicated that both extracts at all concentrations were exhibited a significant antibacterial activity compared to standard drug Gentamicin. The results of this study are similar to that study of *G. senegalensis* extract against methicillin resistant *S. aureus*, *Bacillus subtilis*, *E. coli*, *Salmonella typhimurium* (Garba *et al.* 2018; Jiyil *et al.* 2019). Previous study was indicated that *Q. infectoria* acetone fraction have inhibitory impact on multi-drug resistant *S. aureus*, *Pseudomonus aeruginosa* and *E.coli* (Chusri and Voravuthikunchai 2009; Wan Nor Amilah *et al.* 2014; Abdullah *et al.* 2019). The antibacterial activity of that *Q. infectoria* was higher than that reported by Basri *et al.* 2012 against oral pathogens. Moreover, acetone fractions of two plant extract were proved to exert antibacterial activity against *S. aureus* resulting in 15 mm and/or more growth inhibition zone which is more than that produced by 30 mg/ml gentamycin. The presence of these bioactive compounds such as phenolic compounds also displayed substantial may be responsible for the antimicrobial activities of these plant extracts (Bouarab-Chibane *et al.* 2019).

Conclusion

As per high content of phenolic constituents including tannins and flavonoids, aqueous and alcoholic extracts of the two plant parts tested verified the claimed bioactivity of these plants employed in traditional medicine. Thus; they could possibly be used in preparations to treat inflammation and bacterial infections especially those caused by *S. aureus*. Further studies should be carried out to ascertain the safety and efficacy of *G. senegalensis* and *Q. infectoria* extract being with antioxidant and antibacterial activities.

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