International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182 Print ISSN: 2349-5979
www.allsubjectjournal.com
Volume 2; Issue 11; November 2015; Page No. 665-669

Comparison of antimicrobial activity and thin layer chromatography profile of ethyl acetate fraction of leaves and stem of *Cordia africana* (Boraginaceae)

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Abstract
Cordia africana Lam., belongs to the family Boraginaceae, is native to Angola, the Democratic Republic of Congo, Djibouti, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Malawi, Mozambique, South Africa, Sudan, Tanzania, Uganda, Zimbabwe, Saudi Arabia and Yemen (Warfa, 1988; Friis, 1992). Chloroform and aqueous fractions of leaves and stems *C africana* methanolic were devoid of any activity against all organisms used in this study. Ethyl acetate fraction of leaves showed activity ranged between13 -21 mm. The highest activity was against *S. aureus* while the lowest activity was against *C. albican*. Stems ethyl acetate fraction showed activity between14 -26 mm against all organisms used. The highest activity was against *C. albicans* (26 mm) and the lowest activity was against *A. Niger* and *E. coli.* Thin layer Chromatography of both leaves and stem ethyl acetate fractions was carried out. Different spots with different RF values and colors were observed when plates inspected under UV, 366 nm and sprayed with Para Anisaldehyde.

Keywords: Comparison of Antimicrobial Activity, and Thin Layer Chromatography Profile.

1. Introduction
Antibacterial activities tewolde *et al.* (2013) studied the total phenols of C. africana dry and fresh fruits, and the results were 2317.0 and 264.1mg respectively. Due to the lack of suitable land, most of the forest plantations in Eritrea and Ethiopia are carried out in adverse environments. In Ethiopia, it is widespread in Broadleaved Afromontane Rain Forests, Undifferentiated (Dry) Afromontane Forests ('mixed *Podocarpus* forest') and in reverie forests as well as in the western lowlands (Friis, 1992). C. africana is aindigenous tree that farmers deliberately preserve on their farm, because it improves soil fertility (Mugendi and Nair, 1997). Adverse environments are sites that are marginal for growing economic crops due to their extreme climate (e.g. low rainfall, frost etc.) and poor soil conditions (e.g. nutrient deficient, acid, alkaline soils) (Zobel and Van Buijlene 1989; Evans and Turnbull, 2004). Tewolde *et al*. (2013) studied the antioxidant activity of *C. africana* fruits and it showed weak antioxidant. Whereas, Brime shrimp lethality was domenestrated by Mainen *et al*. (2007) and reveled LC50 at 211.4ug/ml. Hussein (2005) studied the activity of different extracts from *C. african* leaves, bark and roots from different location of Sudan against termite. Ethyl acetate extracts of the different parts showed highest activity. Shelly *et al*. (2012) reported that the leaves pitch showed anti-malarial activity, anti-tuberculosis. Emtinan, *et al*., (2015). The present study was conducted to investigate the antimicrobial activity and phytochemical screening for different parts (leaves, stem, park and fruit) of *C. africana*. The extracts of *C. africana* was screened for its antimicrobial activity against four standard bacteria, two Gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungal strains (*Apergillus Niger* and *Candida albicans*) using the cup plate agar diffusion method. Emtinan, *et al*., (2015) The present study was conducted to investigate the *in-vitro* antioxidant (DPPH assay) and cytotoxic (brine shrimp) of different parts (leaves, stem, park and fruit) of *C. africana*, the different parts of *C. africana* was screened for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant and screened for their cytotoxicity using brine shrimp. The inhibition percentage of antioxidant against (DPPH assay) varied from (37 ± 0.10 to 95 ± 0.00% RSA). The test of cytotoxicity was done using brine shrimp lethality, verified the toxic extracts except stem by water and leaves by methanol and water extracts (Benhura and Chidewe, 2011) studied the content of polysaccharides in *C. african* fruits. The yield percentages of polysaccharides ranged between 1.1 – 2 %.

2. Materials and Methods
Plant materials: Plant samples were collected from its natural habitat from Algalabat (East of Sudan), taxonomically identified and authenticated by Dr. Haidar Abdelgadir at the Herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) where the voucher specimen has been deposited for future work

Preparation of the standard bacterial suspensions
One ml aliquots of a 24-hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 °C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 -10^9 colony forming

units per ml. The suspension was stored in the refrigerator at 4 °C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938; Collee *et al.*, 1996). Serial dilutions of the stock suspensions were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette to the surface of dried nutrient agar plates. The plates were allowed to stand for 2-hours at room temperature for the drops to dry, and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of the colonies per drop (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of stock suspensions, expressed as the number of colony forming unit per ml of suspension. Each time of fresh stock suspension will be prepared, all the above experimental condition will be maintained constant so that suspensions with very close viable count would be obtained.

Preparation of standard fungal organisms

The fungal standard cultures were obtained from the department of Microbiology and Parasitology, Medicinal and Aromatic Plants and Traditional Medicinal Research Institute, Khartoum and were maintained on Sabouraud dextrose agar, incubated at 25ºC for 4 days. The fungal growth were harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline and the suspension was stored in refrigerator till used.

Thin Layer Chromatography

Thin layer chromatography was carried out for the ethyl acetate fractions of stem and leaves, which showed potent activities according the method described by (Stalh 1969)

Stationary phases

The stationary phases used were the following:

A) Silica gel type G
B) Silica gel type GF254

Mobile phases

The following mobile phases were tested:

1. Chloroform : Methanol (8: 2)
2. Butanol : Acetic acid : Water (4:1:5) upper Layer
3. Butanol : Acetic acid : Water (4:1:1)
4. Toluene : Methanol (84:16)
5. Toluene : Ethyl acetate : formic acid (4:5:1)
6. Benzene : Ethyl acetate (80: 20)
7. Butanol : Ethanol: Water (40: 19: 11)

Preparation of plates

30 g of silica gel were shaken with 60 ml distilled water for two minutes using 250 ml stopper conical flask. The slurry was spread using spreader making 0.25 mm thickness on five glass 20×20 cm plates. The coated plates were then allowed to dry at room temperature and activated at 105°C for one hour. The hot plates were stored and allowed to cool down and stored till use.

Application of sample

0.5 g of the ethyl acetate fraction was dissolved in 10 ml ethyl acetate and applied to the plate using capillary tube. Samples were spotted at about 2 cm from the bottom of the plate and 2 cm from each edge. Plate was kept to dry by air and then inserted in tank containing the selected solvent system. After reaching the height of 15 to 20 cm, the plate was taken out of the tank and allowed to air till solvent was completely evaporated. The plate was inspected in day light, then examined under UV lamp and finally sprayed with the specific spraying reagent. RF values of separated compounds which appeared in day light or under UV or after sprayed and heated were calculated as followed:

Distant travelled by spot / distant crossed by solvent front

Preparation of spraying reagents

Reagents were prepared according to method described by (Stalh 1969)

Anisaldehyde-sulphuric acid

Reagent was freshly prepared by dissolving 0.5 ml of anisaldehyde in 50 ml glacial acetic acid and 1 ml conc. sulphuric acid.

3. Results

Table (1): Antimicrobial activity of leaves and stem of *C. africana* fractions of methanolic extracts against Standard Organisms using (50 mg/ml):

Part used	Standard tested organisms* /M.D.I.Z (mm)**					
	B. s	***S. a***	***E. c***	***Ps.a***	***A. n***	***C. a***
Stem	17	21	15	17	15	13
Leaves	19	18	14	15	14	26

Table (2): RF values of separated spots of stem ethyl acetate fraction

Spot	UV 254 nm		UV 366 nm		Para-anizalsehyde		Suggestion
	RF value	Colour	RF value	Colour	RF value	Colour	
1	0.4	Fluoresce	0.4	Fluoresce	0.2	Pale violet	Triterpenes
2	0.5	Fluoresce	0.5	Fluoresce	0.4	Pale violet	Triterpenes
3	0.6	Fluoresce	0.6	Fluoresce	0.5	Violet	Triterpenes
4	0.7	Fluoresce	0.7	Fluoresce	0.9	Blue	Phenol
5	0.8	Fluoresce	0.8	Fluoresce	-	-	-
6	0.9	Fluoresce	-	-	-	-	-

Table (3): RF values of separated spots of leaves ethyl acetate fraction

Spot	UV 254 nm		UV 366 nm		Para-anizalsehyde		Suggestion
	RF value	Colour	RF value	Colour	RF value	Colour	
1	0.4	Fluoresce	0.4	Fluoresce	0.3	Gray	Sugar
2	0.5	Fluoresce	0.5	Fluoresce	0.4	Pale violet	Triterpenes
3	0.6	Fluoresce	0.6	Fluoresce	0.5	Violet	Triterpenes
4	0.7	Fluoresce	0.7	Fluoresce	0.7	Pale violet	Triterpenes
5	0.8	Fluoresce	0.8	Fluoresce	0.8	Green	Sterols
6	-	-	-	-	0.9	Blue	Phenol

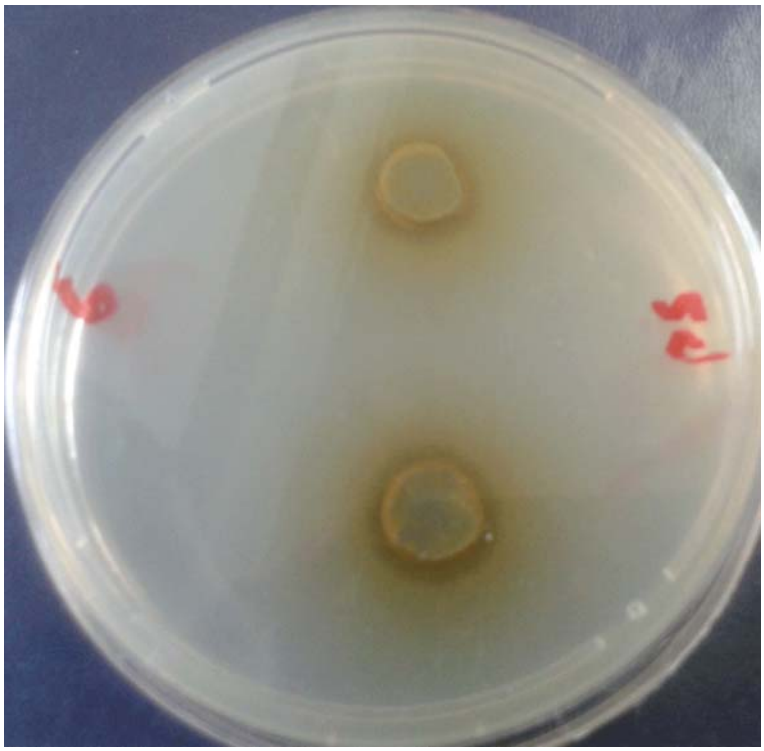
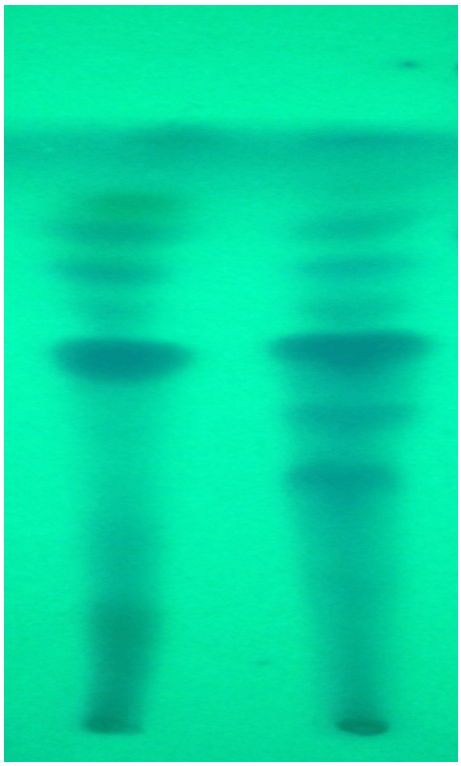


Plate 1: Inhibition zones of ethyl acetate fraction of stems methanolic extract against *Pa. s.*

Plate 2: Inhibition zones of ethyl acetate fraction of stems methanolic extract against *S. a.*



Leaves **stems**

Plate 3: TLC Silica gel (GF_{254} nm) plate showing ethyl acetate fraction of leaves and stems methanolic extracts using Toluene: Ethyl acetate: Formic acid solvent system (48: 60: 6) under UV $_{254}$ nm.

Leaves **stems**

Plate 4: TLC Silica gel (GF_{254} nm) plate showing ethyl acetate fraction of leaves and stems methanolic extracts using Toluene: Ethyl acetate: Formic acid solvent system (48: 60: 6) and Para Anisaldehyde spray reagent.

Leaves **stems**

Plate 5: TLC Silica gel (GF_{254} nm) plate showing ethyl acetate fraction of leaves and stems methanolic extracts using Toluene: Ethyl acetate: Formic acid solvent system (48: 60: 6) under UV $_{366}$ nm.

4. Discussion

Antimicrobial activity of Leaves and stems fractions

Methanolic extracts of leaves and stems, were subjected to liquid – liquid fractionation with chloroform and ethyl acetate and water. Antimicrobial activity of all fractions were carried out. Diameters of inhibition zones for different fractions against standard organisms are shown in Table (1). Chloroform and aqueous fractions of both a leaves and stems methanolic were devoid of any activity against all organisms used in this study. Ethyl acetate fraction of leaves showed activity ranged between13 -21 mm. The highest activity was against *S. aureus* while the lowest activity was against *C. albican*. Stems ethyl acetate fraction showed activity between14 -26 mm against all organisms used. The highest activity was against *C. albicans* (26 mm) and the lowest activity was against *A. Niger* and *E. coli* (Plates 1-2). Thin layer chromatography was done using two types of silica gel (G and GF_{254} nm) and many mobile phases were tested. The best separation was cleared on silica gel (GF_{254} nm) and mobile phase (Toluene: Acetic acid: Ethyl acetate) (48: 4: 60). Leaves fraction showed the presences of five florescence spots with different RF values when inspected under UV lamps (254 and 366 nm). Plated after sprayed with Para anizaldehye spray reagent gave six spots, RF values of separated spots ranged between 0.3 to 0.9 and the colors were gray, violet, bale violet, green and blue. Colors of separated spots indicate that, fraction may contains treterpens, sterols, sugars and phenols. Stem ethyl acetate fraction showed the presences of five florescence spots with different RF values

when inspected under UV lamps (254 and 366 nm). Plated after sprayed with Para anizaldehye spray reagent gave four spots, RF values of separated spots ranged between 0.4 to 0.9 and the colors were gray, violet, bale violet and blue. Colors of separated spots indicate that, fraction may contains treterpens, sugars and phenols. We can say that there were similar compounds (having the same RF values and color) presences in the stem and leaves fraction and this is may caused the activity of both of them.

5. Conclusion

These study from the result it can be concluded that the identified compounds may have many applications like antimicrobial, anticancer and anti-inflammatory. treatment of bacterial and fungal *in vitro* several infections disease.

6. Acknowledgements

We are grateful to Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI). National Center for Research. Khartoum, Sudan.

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