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Comparative Evaluation of Phytochemicals, Antioxidant and Antimicrobial Activity of Four Medicinal Plants Native to Northern Nigeria.

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Abstract: The evaluation of secondary metabolites in four Nigerian native plants; *Cissampelos owarensis, Tacca leontopetaloides, Euphobia hirta* and *Euphobia thymifolia.* Reveals the presence of alkaloids, tannins, saponins, triterpenes, flavonoids, glycosides and carbohydrates. *Cissampelos owarensis* contains all the metabolites while *Tacca leontopetaloides* contain all the metabolites except flavonoids; *Euphobia hirta* contains all the metabolites except anthraquinones. *Euphobia thymifolia* contains all the metabolites except cardiac glycosides. The result of antimicrobial activities of the plants extract on the clinical isolates; *Staphylococcus aureus, Streptococcus pyogene, Salmonella typhi, Escherichia coli, Shigellia dysenteriae, Proteus vulgaris* and *Candida albicans* showed zones of inhibition ranging from 18-27 mm. The result of the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) showed that all the plants' extract inhibited and completely killed *S. aureus* and *E. coli* at a concentration range of 6.25-50 mg/mL and 25-100 mg/mL respectively. The Percentage antioxidant (AA %) activity of the plants extract showed a dose dependant increase, *C. owariensis* had the highest percentage antioxidant activity at 125 µg/mL (91 %) while the least results was recorded for *T. leontopetaliodes* at 125 µg/mL (86 %). The results of the reducing potential and Total Phenolic content express in terms of Gallic acid equivalent (GAE) showed that *C. owariensis* possesses the highest reducing potential and total phenolic content (0.703nm, 12.4 mg), *E. thymifolia* (0.482nm, 7.46 mg), *E. hirta* (0.451nm, 7.00 mg) and *T. leontopetaliodes* showed the least reducing potential (0.217nm, 6.90 mg), when compared with Gallic acid standard (1.268nm). These observations showed that the plants have reducing characteristics hence can act as good antioxidants.

Key word: Medicinal plants, antioxidant, reducing potentials, Total phenolic and antibacterial.

INTRODUCTION

Nature has provided many things for humankind over the years, including the tools for the first attempts at therapeutic intervention. Ancient civilization depended on Plant extracts for the treatment of various ailments. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents. (Kumarasamy *et al.,* 2002) The searches for medicinal plants that are more potent and efficient antibiotic agents have accelerated in recent years. In Nigeria most medicinal plants are traditionally used in folk medicine to treat various diseases, (Alho and Leinonen, 1999) the scientific justification of their uses has not been fully established.

Euphobia thymifolia specie belongs to the genus *Euphorbia* and to the family Euphobiaceae. It is one of the important multipurpose species of desert and arid regions; it provides vegetative cover in dry hot sandy desert areas where little else grows. It contains a crystalline alkaloidal principle allied to quercetrin, it is aromatic, astringent, demulcent and has stimulant and laxative properties. *Euphorbia thymfiolia* has extensive local uses as well as standard application in treatment of bites from poisonous snakes, reptiles, also used in the treatment of diarrhoea and dysentery, helminthiasis, ring worm, skin diseases and leprosy. (Jayaveera *et al.,* 2010).

Euphorbia hirta belongs to the Euphorbiaceae family. It is a small annual herb common to tropical countries (Alabashi *et al.,* 1999). *Euphorbia hirta* is used in the treatment of asthma, worm infestation, inflammation and respiratory tract infection, diuretic, coughs and purgative action. (Igoli, *et al.,* 2005) In

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Nigeria, extracts of the plant are used to treat eye and ear infection and in the treatment of boils, sore and for promoting wound healing. (Burkill, H.M, 1994) Some of the reported phytoconstituents of the plant included triterpenoid, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid while some of the reported scientific uses include it use as an antispasmodic, antiasthmatic, expectorant, anticatarrhal and antisyphilitic. (Adedapo *et al.,* 2005; Falodun, *et al.,* 2006; Trease and Evans, 1989).

Tacca leontopetaloides is a species of flowering plant in the yam family *Taccaceae* that is native to tropical Africa, South Asia, South East Asia, Northern Australia, New Guinea, Samoa, Micronesia and Fiji. The plant is used in the treatment of stomach ailments, diarrhoea and dysentery. It could also be used to stop haemorrhaging and bleeding in the stomach. Apart from these uses, tubers of Polynesian Arrow root contains starch making it an important food source for many pacific island cultures, primarily for the inhabitants of low islands. Also it could be prepared into flour to make a variety of puddings, as well as used to stiffen fabrics and on some islands the stems bast fibres are woven into mats.

Cissampelos is a family that is comprised of about 20 species 13 of which are found in tropical Africa, *Cissampelos owarriensis* roots, bark and leaves are used as *anthelmentic*, *dysmenorrhoea,* sedative, gastro-intestinal complaints such as diarrhoea, dysentery, colic, intestinal worms and digestive complaints and also urogenilla such as menstrual problems, venereal diseases, infertility, to induce contraction of the uterus to start labour or abortion and also to expel the placenta.

In this paper we report the results of our investigation on the comparative antimicrobial and antioxidant activity of these four plants.

MATERIALS AND METHODS

Plant Material:

The plants *Euphorbia hirta, Euphorbia thymifolia, Cissampelos owariensis and Tacca leontopetaloides* were collected from Zaria in September, 2009. The plant were properly authenticated and identified at the Herbarium of the Department of Biological sciences Ahmadu Bello University, Zaria, Nigeria with Voucher numbers 583, 903, 2293, 225, respectively. The plants were air- dried and then pulverized individually, the powdered plant materials (300g) were macerated using methanol. The various extracts were concentrated using a rotary evaporator under reduce pressure at 40^{o}C and then air dried.

The extracts were tested for the presence of alkaloids, flavonoids tannins, carbohydrates saponins cardiac glycoside, anthraquinones, sterols and triterpenoids. These tests were performed using methods described by Trease and Evans (1989) and Sofowora, (1999).

Biological studies:

The antimicrobial activities of the methanol extracts from the plants, *E. hirta, E. thymifolia C. owariensis,* and *T. leontopetaloid* were determined using microbial strains obtained from the Department of medical microbiology, Ahmadu Bello University Teaching hospital Zaria, Nigeria (ABUTH); They include Gram-positive bacteria: *Staphylococcus aureus, Streptococcus pyogene,* the Gram-negative bacteria; *Escherichia coli, Salmonella typhi, Shigellia dysenteriae, Proteus vulgaris* and the fungi *Candida albicans*. The cork and bore diffusion method of Karou *et al* (2006), was used to determine the antibacterial activity of the extracts. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar (MERCK) and incubated for 24h at 37^{0}C. about 5 discrete colonies were aseptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 MacFarland Standard. The suspensions were then inoculated on the surface of sterile Mueller – Hinton Agar plates using sterile cotton swabs. A sterile 6mm diameter cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with different concentration of the extract and polyphenolic fraction. The plates were incubated for 24h at 37^{0}C. All the tests were performed in triplicate and the antibacterial activities were determined as mean diameters of inhibition zone (mm) produced by the plant extracts.

Minimum Inhibitory Concentarion (MIC):

The minimum inhibition concentration (MIC) were determined for the medicinal plant extracts showing antimicrobial activity in the cork and bore diffusion assay, using micro broth dilution method in accordance with NCCLS, (2002). Serial dilution of the least concentration of the extracts that showed activity were prepared using test tubes containing 9ml of double strength nutrient broth (OXOID). The test tubes were inoculated with the suspension of the standardized inocula and incubated at 37^{0}C for 18h. Minimum inhibition concentrations (MIC) were recorded as the lowest concentrations of the extracts showing no visible growth (turbidity) in the broth.

Minimum Bactericidal Concentration (MBC):

The minimum bactericidal concentration were determined by aseptically inoculating aliquots of culture, from the minimum inhibition concentration (MIC) tubes that showed no growth, on sterile nutrient Agar (OXOID) plates and incubated at 37^0C for 48h. The MBCs were recorded as the lowest concentration of extracts showing no bacterial growth at all.

Dpph Radical Scavenging Activity:

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was carried out according to the method described by Mensor *et al*., (2001). Different concentrations of the test sample and standard (Gallic Acid and Ascorbic Acid) were prepared; 1000µg/mL, 500µg/mL, 250µg/mL, 125µg/mL, 62.5µg/mL respectively. DPPH solution (1.0 mL, 0.3M) was added to methanol solution of plant extract and standard (2.5 mL) and incubation for 20 min. at room temperature in the dark. The absorbance of the resulting mixture was measured at 518 nm using Hewlett Packard UV-Vis spectrophotometer (UV-Vis model 1601, Shimadzu, Kyoto, Japan) and the percentage Antioxidant activity (AA %) was calculated using the expression below:

$$AA\% = 100 - \frac{\{\text{Abs Sample} - \text{Abs blank}\}}{\text{Abs Control}} \times 100$$

The scavenging reaction between DPPH and an antioxidant (H-A) can be written as: -

DPPH + H-A → DPPH – H + ($A^{\cdot}$)
Purple yellow

Determination of Reducing Potential:

This was determined according to the method of Blois, (2001) . The plant extract (1.0 mL, 250 μgmL^{-1}) was mixed with 2.3 mL phosphate buffer (0.2 M, P_H 6.6) and 2.5 mL of 1% potassium ferricyanide (K_3 [Fe CN_6]). The mixture was incubated at 37^0C for 20 min. 10% Trichhloroacetic Acid (2.5 mL) was added to the mixture then centrifuged for 10 min at 1000 rmp, the supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. After standing for 10 min, the absorbance was measured at 700 nm using Hewlett Packard UV-Vis spectrophotometer.

Determination of Total Phenolics:

The total phenolics in the extract were determined using Folin-Ciocalteu method as described by Kujala *et al,* (2000). To each sample solution (1.0 mL) and the standard (Gallic Acid) was added 5 mL of folin-ciocalteu and 4 mL Sodium carbonate (7% w/v) and shaken. The solution was allowed to stand for 30 min. in the dark at room temperature, after which absorbance was measured at 765nm using a spectrophotometer. The amount of total phenolics was expressed as Gallic acid equivalent (GAE) in milligram per gram dry plant.

RESULT AND DISCUSSION

The evaluation of secondary metabolites (Table 1) showed that *C. owarensis* contains; alkaloids, tannins, saponins, triterpenes, flavonoids, glycosides and carbohydrates. *T. leontopetaloides* contain all the metabolites except flavonoid while *E. hirta* contain all the metabolites except antraquinone. *E. thymifolia* contain all the metabolites except cardiac glycoside. The results of determination of zone of inhibition of all the plants extract (Table 2) ranges from 18-27 mm against the test organisms. *E. hirta* was observed to be the most active with zone of inhibition of 27 mm against *S. aureus*, while *E. thymifolia* showed a zone of 21 mm against *S.aureus. S. aureus* and *E. coli* were sensitive to all the plants extract, while *S. pyogenes* showed sensitivity to all except *T. leontopetaloides. S. dysentariae* was observed to be sensitive to all the plant extracts, except *E. hirta. P. vulgaris* was observed to be only sensitive *to E. thymifolia. Candida albicans* was only sensitive to *E. thymifolia* and *C. owarensis*. The result of the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) shown in Table 3,4 showed that all the plant extracts inhibited and completely kill *Staphylococcus aureus* at various concentration; *C. owarensis* (MIC=12.5 mg/mL, MBC= 50 mg/mL), *T. leontopetaloides* (MIC= 25 mg/mL, MBC= 100 mg/mL), *E. hirta* (MIC=6.25 mg/mL, MBC= 50 mg/mL) and *E. thymifolia* (MIC= 12.5 mg/mL, MBC= 100 mg/mL). The MIC of the plants on *E. coli* is; *C. owarensis* (MIC= 12.5 mg/mL, MBC= 100 mg/mL), *T. leontopetaloides* (MIC=12.5 mg/mL, MBC= 50

mg/mL), *E. hirta* (MIC= 25 mg/mL, MBC= 100 mg/mL) and *E. thymifolia* (MIC= 25 mg/mL, MBC= 100 mg/mL). The result of the DPPH Radical scavenging activity of the plants extract (Table 5, 6, Fig.1) showed a dose dependant increase in percentage antioxidant activity (AA %). C*issampelos owariensis* has the highest percentage antioxidant activity at concentrations of 125 µg/mL (91.0 %), 250 µg/mL (96.0 %), 500 µg/mL (97.0 %) and at 1000 µg/mL (97.9%). This compared favorably with the results of Gallic acid standard used at the same concentration. The least result was recorded for *Tacca leontopetaliodes* at concentrations of 125 µg/mL (86.0 %), 250µg/mL (87.0 %), 500 µg/mL (89 .0%) and at 1000 µg/mL (93.0 %).

The results of the reducing potential of the standard (Gallic acid) and the plants extract (Table 7, Fig. 2) showed that *C. owariensis* possesses the highest reducing potential (0.703 nm) followed by *E. thymifolia* (0.482 nm) and then *E. hirta* (0.451 nm). Whereas *Tacca leontopetaliodes* showed the least reducing potential (0.217 nm), as compared with Gallic acid standard (1.268 nm). These observations showed that the plants have reducing characteristics hence can act as antioxidants, reducing agent, hydrogen donator and oxygen quenchers (NCCLS, (2002).

Table 1: Results of phytochemical screening of the plant extracts

Phytochemical	Test	Methanol extract			
		C. owarensis	*T. leontopetaloides*	*E. hirta*	*E. thymifolia*
Alkaloids	Dragendoff	+	+	+	+
	Wagner	+	+	+	-
	Picric acid	+	+	-	+
	Tanin acid	+	+	+	-
	Meyer	+	+	-	+
Tannins	$FeCl_3$	+	-	-	-
	PbOAc	+	+	+	+
Flavonoids	Shinoda	+	-	+	+
	NaOH	+	-	+	+
Saponins	Froting	+	+	+	-
Triterpenes		+	+	+	+
Carbohydrates	Molish	+	+	+	+
	Red. Sugar	+	+	+	+
Glycosides	Cardiac	+	+	+	-
	Antraquinone	+	+	-	+
	Kadde	+	+	+	+
	Cardenolides	+	+	+	+

Key: + = present, – = absent

Table 2: Results of zone of inhibition of the plant extracts (mm)

Test Organism	*E. hirta*	*E. thymifolia*	*T. loentapetaloides*	*C. owarrensis*
S. aureus	27	21	18	23
S. pyogenes	22	24	00	20
S. typhi	00	00	20	00
E. coli	18	20	21	20
S. dysenteriae	00	19	19	19
P. vulgaris	00	17	00	00
C. albicans	00	19	00	19

Table 3: Results of Minimum inhibition concentration (mic) of the extracts (mg/mL)

Test Organism	*E. hirta*	*E. thymifolia*	*T. leontopitoloides*	*C. owarrensis*
S. aureus	6.25	12.5	25	12.5
S. pyogenes	12.5	12.5	ND	12.5
S. typhi	ND	ND	12.5	ND
E. coli	25	12.5	12.5	12.5
S. dysenteriae	ND	25	25	25
P. vugaris	ND	25	ND	ND
C. albican	ND	25	ND	25

Table 4: Results of Minimum bactericidal concentration (mbc) of extracts (mg/mL)

Test Organism	*E. hirta*	*E. thymifolia*	*T. leontopitoloides*	*C. owarrensis*
S. aureus	50	100	100	50
S. pyogenes	100	50	50	50
S. typhi	ND	ND	50	ND
E. coli	100	100	50	100
S. dysenteriae	ND	100	100	100
P. vugaris	ND	100	ND	ND
C. albican	ND	50	ND	50

KEY: ND = not determine

Table 5: Result of the Absorbance of DPPH Assay (nm)

	Concentration (µg/L)				
Sample/Standard	62.5	125	250	500	1000
Gallic acid	0.085±0.01	0.070±0.04	0.070±0.02	0.060±0.03	0.057±0.02
E. hirta	0.195±0.02	0.180±0.02	0.138±0.01	0.147±0.15	0.185±0.01
E. thymifolia	0.791±0.09	0.378±0.09	0.211±0.20	0.146±0.06	0.155±0.02
C. owarrensis	0.541±0.02	0.261±0.08	0.060±0.01	0.059±0.08	0.042±0.23
T. leontopetaloides	0.359±0.08	0.291±0.07	0.461±0.08	0.557±0.08	0.194±0.05

Table 6: Result Of The Precentage Antioxidant Activity(AA%)

	Concentration (µg/L)				
Sample/Standard	62.5	125	250	500	1000
Gallic acid	91	92	93	95	100
E. thymifolia	82	89	90	91	93
E.hirta	23	64	81	90	93
C. owarrensis	60	91	96	97	98
T. leontopetaloides	78	86	87	89	93

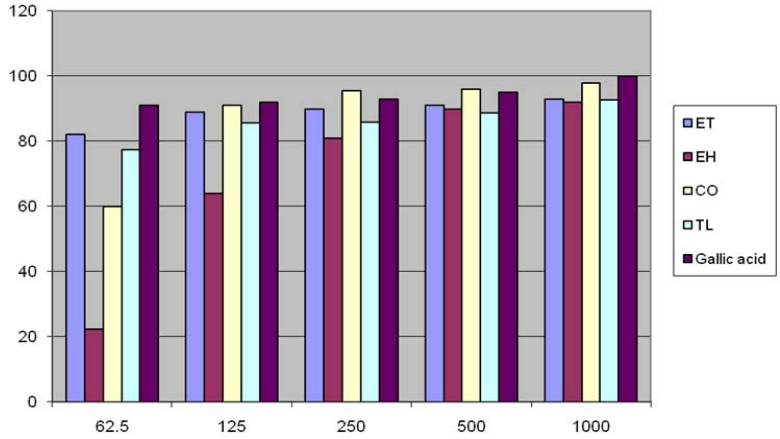

Fig. 1: Graph of percentage Antioxidant activity of the extracts and Standard
KEY: ET=*Euphorbia thymifolia, EH=Euphorbia hirta, CO=Cissampelos Owarrensis, TL=Tacca leontopetaloides*

Table 7: Results of Reducing Power Determination

Sample/Standard	62.5	125	250	500	1000	Mean±SD
Gallic acid	0.047	0.104	0.418	2.636	3.133	1.268±1.641
E. hirta	0.162	0.403	0.488	0.621	0.736	0.451±0.219
E. thymifolia	0.144	0.424	0.073	0.879	0.879	0.482±0.355
C. owarrensis	0.058	0.341	0.393	1.000	1.725	0.703±0.666
T. leontopetaloides	0.073	0.174	0.224	0.212	0.403	0.217±0.119

The result of the total phenolic assay of the plants extracts express in terms of Gallic acid equivalent (GAE, Table 8, 9) showed that *C. owariensis* has the highest total phyenolic content (12.4 mg), this was followed by *E. thymifolia* (7.46 mg), *Euphorbia hirta* (7.00 mg), *Tacca leontopetaloides* showed the least phenolic content (6.90 mg). From the results it can be inferred that a relationship exist between the phenolic content of the plants and the antioxidant activity such that as phenolic content increases the radical scavenging activity also increases.

Table 8: Total phenolics determination; Absorbance Of Gallic Acid Standard At 700nm.

Conc. (mg/mL)	1	2	3	Mean±SD
0.025	0.074	0.031	0.076	0.060±0.03
0.050	0.311	0.239	0.233	0.261±0.04
0.100	0.702	0.695	0.676	0.691±0.01
0.200	1.504	1.520	1.538	1.521±0.02
0.400	2.398	2.386	2.261	2.348±0.08

Table 9: Result Of The Mean Absorbance And The Extrapolated Concentration From The Calibration Curve

Sample	Absorbance	Extrapolated conc.	GAE
E. thymifolia	1.020±0.21	0.150	7.46
E. hirta	0.993±0.16	0.146	7.00
C. owarrensis	1.119±0.05	0.169	12.4
T. leontopetaloid	1.008±0.17	0.149	6.90

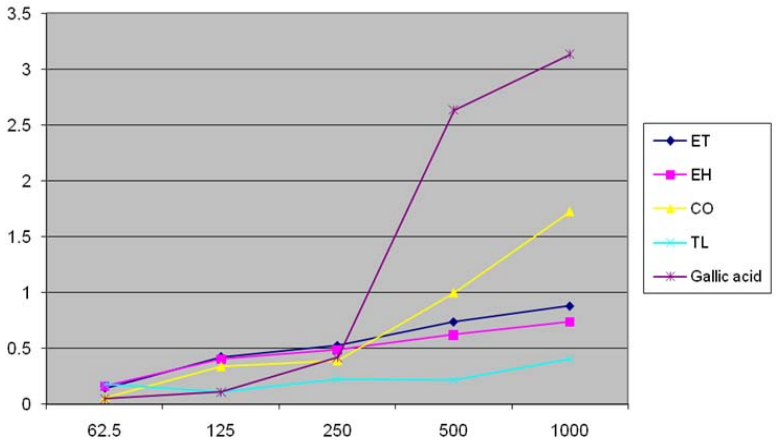

Fig. 2: Reducing Power Determination of the extract and the gallic acid standard
KEY: ET=*Euphorbia thymifolia, EH=Euphorbia hirta, CO=Cissampelos Owarrensis, TL=Tacca leontopetaloides.*

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