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Phytochemicals, Antimicrobial, Antioxidants and Cytotoxicity properties of the Ethanolic Extracts of *Commiphora africana and Butryospermum paradox*

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Abstract
The study was undertaken to evaluate the in vitro antioxidant, anti-microbial and cytotoxicity properties of methanolic extract of leaves of the plant Commiphora *africana* and *Butryospermum paradox* with a view to exploring the therapeutic potentials of the plant especially as far as treatment and management of oxidative, microbial and other related disorders are concerned. The study involved collection, identification, extraction of *Commiphora africana* and *Butryospermum paradox* leaves with ethanol. Methanolic extract of leaves of *Commiphora africana* and *Butryospermum paradox* were prepared and analyzed qualitatively for presence or absence of various phytoconstituents such as Saponins, Tannins, Flavonoid, Alkaloid, Essential oils, Glycosides, Phenols, Terpenes and Protein. Evaluation of the antioxidant and anti-microbial potentials of the plant using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide reduction and disc diffusion method were also carried out. The plants exhibited potent and significant antioxidant and anti-microbial activities which are concentration-dependent, as well as appreciable thrombolytic activities. Moreover, the extracts compared favorably with various reference drugs used in the study. It was observed that the extract possessed bioactive components with beneficial effects in the management of oxidative, microbial and other related conditions. Presence of various phytochemical compounds in the leaves of *Commiphora africana and Butryospermum paradox* justifies the medicinal use of the plants. The present study results could provide future insight of this plant to be useful in pharmaceuticals for as a cure for various diseases.

Keywords: Anti-microbial, Alkaloid, Cytotoxicity, Commiphora *africana*, Methanol

Introductions

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Ladan *et al*., 2011). Traditional use of medicine is recognized as a way to learn about potential future medicines. In present days, almost all pharmacopoeias in the world prescribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany) that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine).

For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent (Banjor and Farokhi, 2004). Although it has been estimated that about one in four of all prescribed drugs' and almost 7,000 different medicaments contain compounds of plant origin or their

derivatives with their commercial value being put at about $40 billion annually indicated that about 33%of drugs produced in the developed countries are derived from plants (Carter *et al*., 2000). *Butryospermum paradox* and *Commiphora africana* are very cheap and available plant mostly found in West Africa. A prove of their use for medicinal purpose is investigated by assessing the composition of their phytochemicals, antioxidants and their ability to cause cytotoxicity to brine shrimps.

Materials and Methods

Materials and Reagents

Methanol, Soxhlet apparatus, rotary evaporator, filter papers, freeze dryer, n-hexane, ethyl acetate, DPPH, H_2O_2, etc.

Sample collection and preparation

Selection of plants for the study for secondary metabolite constituents was based on the research on ethnomedicinal survey of traditional medicine of Lala people of Adamawa state, Nigeria. (Kubmarawa *et al*., 2013).

Plant materials were collected from Lala district of Gombi Local Government Area of Adamawa State. Moreover, the samples were identified and authenticated by Dr. Bristone Basiri of school of Life Science, Department of Plant Sciences Modibbo Adama University Yola. During the collection of the plant, it was kept in mind that the specimen to be studied should be healthy, since microbial and other infection may change the metabolites produced by the specimen. e.g. phytoalexin formation. Variation in collection site altitude. plant age, climate, and soil type can influence the concentration level of secondary metabolites and even kinds of compounds biosynthesized in certain cases this was also kept in mind (Kinghorn, 2001). In this work, fresh parts of roots of plants were used.

Methods

Extraction of Plant material and Isolation

The air-dried and powdered plant materials (50 g of each) was extracted with 500 mL of ethanol with Soxhlet apparatus for 3 hours each. The ethanolic extracts obtained were concentrated using a rotary evaporator (Buechi, Switzerland) and freeze dryer to give the crude dried extract of the samples. Moreover, a portion of ethanolic crude extract was dissolved in water to obtain an aqueous extract. The aqueous extract was successively partitioned with n-hexane, Petroleum ether, ethyl acetate and butanol to obtain hexane, petroleum ether, ethyl acetate and n- butanol fractions. the ethanol crude extract and its fractions were stored at 4°C till usage for phytochemical, biological assays and isolation of active compounds.

Screening the Extracts for Bioactive Agents

Phytochemical screening for the major constituents of the plant extracts was carried out using standard qualitative methods as described by various authors and adopted by (Kubmarawa *et al*., 2007)

Determination of Antimicrobial Activity

The antimicrobial activity of the extract was determined using Disc Diffusion Method (Ravi et a1., 2010). Petri plates containing 10ml of Mueller Hinton agar medium was seeded with 24 hours old culture of a selected bacteria strain. Sterile filter paper disc (9mm in diameter) containing 1000-5000 ppm of a plant extract dissolved in ethanol, which was allow to dry off and placed on the medium. Moreover, the disc alone served as negative controls. A standard disc containing chloramphenicol antibiotic drug (30 ug/disc) was as a positive control and Incubation was done for 14 hours at 37°C (Swamy *et al*., 2016). The assessment of antimicrobial activity based on the measurement of diameter of incubation zone formed around the disc (diameter of inhibition zone minus diameter of the disc). An average zone of inhibition was calculated for three replicates. An inhibition zone of 8mm or greater was considered as a good antimicrobial activity (Ali *et al*., 2001). According to Ogunwande, 2001 a cleared zone > 10 mm was interpreted as sensitive while < 9 mm was interpreted as resistance.

Determination of Anti-Oxidant Activity of plant materials.

Quantitative 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay.

The quantitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity of the plant extracts. The purple color, typical for free DPPH radical fades, and the change in absorbency at $\lambda = 517$ nm is measured spectrophotometrically.

The method was carried out as described previously by Kubmarawa *et al*. (2013) and adopted by Runde *et al*. (2015), (Usunomena and Chinwe, 2016). The extracts were dissolved in methanol, and various concentrations (2, 6, 12, 24, and 50µL/mL) was used. The assay mixture contained in a total volume of 1 mL, 500µL of the extract, 125µL prepared DPPH (1mM in methanol), and 375µL solvent (methanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation below: % or radical scavenging = [(Abs control – Abs Sample) ÷ Abs control] x 100 (Runde *et al*., 2015).

Hydrogen peroxide scavenging activity

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (20-500 µg/ml) in methanol will be added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contained the phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging will be calculated as:

H_2O_2 scavenging effect (%) = A control – A sample / A control × 100

where A control is the absorbance of the control, and A sample is the absorbance in the presence of the sample or standards (Ruch *et al*., 1989).

Cytotoxic brine shrimp assay

Brine shrimp lethality test

Brine shrimp lethality bioassay, was carried out to investigate the cytotoxicity of plant extracts. 50 mg of Artemia salina (Leach) eggs was added to a hatching chamber containing Ocean / Sea water (75 ml). The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. 20mg of test fractions F1, F2, F3, F4, F5 and F6 of the various plant species were separately dissolved in 2 ml of methanol, form this, 500, 50, and 5µl of each solution was transferred into vials corresponding to 1000, 100, and 10µg/ml, respectively. Each dosage was tested in triplicate. The vials (9 per test fraction) and one control containing 500µl of solvent will be allowed to evaporate to dryness in about 48h at room temperature.

Forty-five milliliters (45 ml) of Ocean/Sea water were added to each vial, and 10 larvae of A. salina Leach (taken 48 – 72 h after the initiation of hatching) was added to each vial. The final volume of solution in each vial was adjusted to 5ml with Ocean/Sea water immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. LC50 values were determined at 95 % confidence intervals by analyzing the data on a computer loaded with a "Finney Program" The LC50 values of the brine shrimps obtained for extracts of the plants studied were recorded. (Adoum 2009). After 24 h of incubation survivors were counted with help of 3× magnifying glass and calculation was done using Abbot's formula; % Death = (Sample-control/control) × 100.

Results and Discussions

Basic Phytochemical Screening of Plants

The phytochemical investigation of ethanolic extract of two medicinal plants is shown in Table 1. Below. Glycosides, Phenols, Flavonoids, Tannins, Terpenoids and proteins were found to present in all the ethanolic extracts of leaves, stem, bark, root and arial part of *Butryospermum paradox* and *Commiphora africana*. Moreover, Alkaloid, Saponins and Essential oils were only present in *Butryospermum paradox*. This work goes in line with the work of Akor and Anjorin (2009), which studied the phytochemicals of *Commiphora africana* root extracts.

Table 1: Qualitative phytochemical analysis

Phytochemicals	***Butryospermum paradox***	***Commiphora Africana***
Saponins	+	-
Tannins	+	+
Flavonoid	+	+
Alkaloid	+	-
Essential oils	+	-
Glycosides	+	+
Phenols	+	+
Terpenes	+	+
Protein	+	+

Note: + = Trace of the compound, ++ = Moderate constituent, +++ = Large Constituent and ND = Not detected

Anti-oxidant Activities of Butryospermum paradox and Commiphora africana plants

DPPH method.

The free radical scavenging activity of the medicinal plants of *Butryospermum paradox* and *Commiphora africana* were obtained using 2, 2 – Diphenyl-1-picrylhydrazyl. Moreover, the percentage Scavenging capacity for each medicinal plant and that of the standard (Ascorbic acid) were measured at varying concentrations (5, 10, 15, 20 and 25 µL). From the result all the samples have exhibited antioxidant activity as shown in table 1. Furthermore, All the two plant extracts showed a dose dependent scavenging activity DPPH comparable to standard antioxidant. In DPPH assay the antioxidant activities of the *Butryospermum paradox* ranged between 34.28 to 92.34 % and *Commiphora africana* ranged between 48.54 to 95.74 % and the control ranged between 43.65 to 98.23 % which showed lower activities than the two plants with IC_{50} of 3.20 and 4.37.

These antioxidant activities can be explained by the presence of metabolites notably, including the total phenolics and flavonoids found in *Butryospermum paradox* and *Commiphora africana* extracts and some of the identified compounds. The significant correlation of phenolics to the biological activity conformed with previous data that indicated the important contribution of phenolics and flavonoids (Compaor´e M *et al*., 20011, Lamien-Meda A *et al*., 2008, Aravindaram K 2010). Thereby, the anti-DPPH, anti ABTS and anti H202 activities have beendemonstrated in a related work (Lee JH *et al*., 2010, Bellik Y *et al*., 2012, Shahidi F, Ambigaipalan P. 2015). The antioxidant and anti-inflammatory properties of these plants could justify its importance in the fight against oxidative stress diseases and its corollaries.

Table 2: Showing results of Antioxidant activities using 2, 2 – Diphenyl-1-picrylhydrazyl (DPPH) method.

Plant Extract	**5µl**	**10µl**	15**µl**	**20µl**	25**µl**	**IC_{50} µl**
Butryospermum paradox	34.28	51.22	68.93	81.45	92.34	3.20
Commiphora africana	48.54	66.54	73.76	84.78	95.74	4.37
Control (Ascorbic acid)	43.65	68.63	77.98	88.71	98.23	2.33

Hydrogen peroxide method.

The scavenging effect of the plant extracts on the peroxide free radicals were expressed as % inhibition and they were compared with standard antioxidant, ascorbic acid. The results of antioxidant activities (H_2O_2) of the various plants are presented in table 2. Hydrogen peroxide is involved in the generation of hydroxyl radicals which can initiate cytotoxicity. Therefore, any substance that can remove H_2O_2 will protect the living system (Al-Owaisi *et al*., 2014). The results of free radical scavenging activity by H_2O_2 method also showed a concentration dependent activity. All the extracts scavenged H_2O_2 probably by donating electrons to the hydrogen peroxidase, thereby converting it into water. The hydrogen peroxide scavenging activity of prepared extracts were found in the following order of *Commiphora africana* greater than *Butryospermum paradox*. The maximum antioxidant activities by H_2O_2 assay were observed at 25µL while the lowest were observed at concentration 5µL. The % inhibition produced by ascorbic acid at concentration of 25µL was greater than the scavenging activities of each extracts at a concentration of 25µL. Thus, it can be concluded that the antioxidant activity of these

plants is due to the presence of phytoconstituents and is consistent with the work of (Al-Owaisi *et al*., 2014). With the above order the IC_{50} ranges from (2.25-2.60 IC_{50}) for *Butryospermum paradox* and Commiphora *Africana* .

Table 3: Result of Antioxidant activities of ten different medicinal plants using Hydrogen peroxide method.

Plant Extract	**5µl**	**10µl**	15**µl**	**20µl**	25**µl**	**IC_{50} µl**
Butryospermum paradox	24.28	36.22	48.03	61.45	74.64	2.60
Commiphora africana	34.64	46.34	57.71	63.98	78.84	2.25
control (ascorbic acid)	43.55	56.93	74.78	83.61	92.59	2.50

Antimicrobial activities of medicinal plants

The anti-bacterial activity of *Butryospermum paradox* and *Commiphora africana* plants against six microbes is summarized in Table 4. below. The antimicrobial activities of two plants were investigated using disc diffusion method against five selected microorganisms, namely *Klebsiella pneumonia, Escherichia coli*, Muti-drug resistant *Acinetobacter, Salmonella typhy, Candida albicans and Pseudomonas aeruginosa*. The results revealed that the selected plants showed antibacterial activity with varying magnitudes. The zone of inhibition above 7 mm in diameter was taken as positive result. Generally, most of the tested organisms were sensitive to some of the plants. All the two plants tested showed antibacterial activity against two or more microorganisms. *Commiphora africana* showed maximum activity against *Klebsiella pneumonia, Salmonella typhy* and Muti-drug resistant Acinetobacter species while *Butryospermum paradox* showed maximum activity against Muti-drug resistant Acinetobacter and E. coli. On the other hand, *Commiphora africana*, *Butryospermum paradox* and DMSO doesn't have inhibition over candida albicans and Pseudomonas aeruginosa. Both gram-positive and gram-negative bacteria were sensitive to the *Commiphora africana.* There was no inhibition growth with the control (DMSO). Phytochemical are non-nutrient bioactive natural compounds that are produce by plants as secondary metabolites and are also very good antimicrobial agents. (Anyawun and Okoye, 2016).

Commiphora africana and *Butryospermum paradox* extracts have antimicrobial activity in which all extracts showed reasonable activity. This is in line with the finding of Toma *et al*. (2016) where the gram-negative bacteria had least activity, it also concurrent with previous studies by which showed that *Commiphora Africana* species have considerable antimicrobial activity. Akor and Anjorin (2009) reported the effect of root ethanolic extract from *Commiphora africana* to be active against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Musa (2008) also showed in his work that *Commiphora kerstingii* stem bark contains antimicrobial activities against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens and Bacillus subtilis*. It was observed that *S. aureus* had minimum value for the ethanolic extracts, while *E. coli* only had minimum for Petroleum ether extract, 500µg/ml, which was higher than all concentrations used.

The antimicrobial activity could be as a result of the presence of terpenoids and flavonoids which were most abundant in the *Commiphora africana and Butryospermum paradox* extracts. Terpenoids have been reported to influence cell membrane structures by increasing membrane fluidity and permeability, changing the topology of membrane proteins and inducing disturbances in the respiration chain of a microbial pathogen (Sieniawska *et al*., 2017). Flavonoids have been associated with inhibition of cytoplasmic membrane functions and DNA gyrase enzyme (Cao *et al*., 2019). These properties may explain why *Commiphora Africana* and *Butryospermum paradox* crude extracts had high antimicrobial activity.

Table: 4. Showing Antimicrobial Activities of two Medicinal plants.

Plant Extract	***Klebsiella Pneumonia***	***Multi-drug resistant Acinetobacter***	***E.Coli***	***Salmonella Typhi***	***Candida albicans***	***Pseudomonas Aeruginosa***
Butryospermum paradox	_	+	+	_	_	_
Commiphora africana	+++	+	++	++	_	_

Toxicity test using brine shrimps

In this study, LC_{50} values of *Butryospermum paradox*um and *Commiphora africana plants* ethanolic extract were 4180 and 9819 respectively. From the observed lethality of the selected plant extracts to brine shrimps indicated the presence of potent cytotoxic and probably anti-tumour components of these plants. The brine shrimp lethality of the two tested samples for ethanolic extract was found to be concentration-dependent. The ethanolic extract of the plants showed direct relationship with the mortality rate of the brine shrimp because as the concentration of the plant extracts increases, the value of brine shrimp mortality after the stipulated hours also increases.

Cytotoxicity studies with normal cell culture have not been studied extensively on plant extracts and this is vital for safety. In a similar study, Vero cells were and they were considered safe when CC50>20 µg/mL (Kigondu *et al*., 2009; Zirihi *et al*., 2005). Also, in a related work Hexane extract of *Commiphora Africana* and *Butryospermum paradox*a showed cytotoxic effects with an Inhibitory concentration of 4.23 µg/mL (Charity Mwangi *et al*., 2020). Moreover, the brine shrimp study also goes in line with the work of Meyer et. al., 1982, (Moshi (Moshi *et al*., *et al*., 2006; Abou-Shoer *et al*., 1988; Kupchan *et al.,* 1980), *Phyllanthus engleri* (Moshi et al., 2006; Ratnayake et al., 2009) *and Ximenia americana* 2004; Asres *et al*., 2001.

Table 5: Cytotoxicity test of ten different plant extracts using brine shrimp's assay**.**

Extracts	Conc. ppm	No. Nauphli	6HR	12HR	24HR	MEAN	% Mortality	LC_{50}
Commiphora africana	10	18	2	4	5	4	22	9819
	100	20	3	5	6	5	25	
	200	19	5	6	8	6	32	
	500	16	5	7	7	6	38	
	1000	20	7	8	10	8	40	
*Butryospermum paradox*um	10	20	1	1	3	2	10	4180
	100	18	0	2	5	2	11	
	200	20	2	5	8	5	25	
	500	19	2	8	12	7	37	
	1000	19	4	7	9	7	37	

Conclusion

The phytochemical results show that plant has naturally occurring phytochemical which have various application. The result of antioxidant activities of the two plants extract using DPPH, H_2O_2 and FRAP exhibited a great radical scavenging when compared to the antioxidant of ascorbic acid available in the market.

This study is a phytochemical, antioxidant and cytotoxicity activities of *Butryospermum paradox*a and *Commiphora africana* plants. It indicates that the plants have the potential to generate metabolites that can be used as supplements, since it contains valuable phytochemicals. Some of the crude ethanolic extracts demonstrated good antioxidant, antimicrobial activity which could result in the discovery of novel drug for the treatment microbes. Moreover, the high toxicity

exerted by the extracts on brine shrimp lethality bioassay suggests bioactive principles in the plant as claim by the traditional users.

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