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Research Article

Evaluation Antioxidant and Antibacterial Activities of n-Butanol Fraction of *Conocarpus Erectus* L. Leaves Extract

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ABSTRACT

N-butanol extract of *Conocarpus erectus* L. leaves was studied for antioxidant and antibacterial activities. *In vitro* systems for example, 1, 1- diphenyl-2 picrylhydrazyl (DPPH) radical, and antioxidant capacity (Ferric Reducing Antioxidant Power, FRAP assay). The antibacterial activity was determined using the hole-in-plate agar diffusion. The extract scavenged DPPH radicals comparable with ascorbic acid and butyl hydroxytoluene (BHT). The n-butanol extract of *Conocarpus erectus* L. leaves showed high free radical scavenging activity toward DPPH radical between (29.87-79.33). The n-butanol extract had radical scavenging activity less than the Ascorbic acid (34.61-80.76) and butyl hydroxytoluene (BHT) (33.33-66.66). The results indicated the antioxidant capacity of butanol extract had a significantly (0.50-1.11nm) much higher than ascorbic acid (0.5-0.86nm) and butyl hydroxytoluene (BHT) (0.30-0.76nm). Antibacterial activity was found to be present in n-butanol extract of *Conocarpus erectus* L. leaves being highest in *Straptococcus* (22mm). Zones of inhibition for *E.coli*, *Staph. aureus* and *Enterobacter* were 21, 20 and 19 respectively. Results show plants to possess antibacterial activity.

1. Introduction

Natural products assume an imperative part in the treatment of various infections. The history of utilization of plants to various conditions is extremely old. The foremost records discovered demonstrate that plants have been utilized in Mesopotamia and Egypt thousands of years ago. Various phytochemicals have been secluded from various plants which are presently being recommended via medical experts all around a globe**[1,2]**. As indicated by the World Health Organization (WHO) the medicinal plants are every plant which, in one or more of its structures, contains elements that may be utilized for helpful purposes, or which are forerunners for chemo-pharmaceutical semi synthesis**[3]**. The therapeutic plants may either be the wild or the developed plant particularly for restorative purposes. As there are broad homegrown pharmaceuticals accessible in the present time, the alike material may be characterized in various structures, for example the powered plant material may be measured as both a natural material and/or herbal preparation and when it's accessible in a form of the packing material, it's considered as homegrown restorative product**[4]**. In every an herbal preparations, it's easy to systematize in terms of a defined amount and concentration of an active components, if the medicinally active constituents have been recognized and are notable. The herbal drugs are the more extensive term which embraces herbs, herbal preparations, and materials counting an ended herbal product**[5]**. The expanding utilization of natural medications has made their utilization a general health issue, because of the likelihood of getting to products without quality. The proficiency and safety must be approved through ethno pharmacological studies, certification and technical and scientific quality to be accomplished by controlling a raw materials, ended products, bundling materials, pharmaceutical formulation and constancy studies**[14,4]**. Antioxidants are a body's natural protection instruments against the harming impacts of "free radicals" and oxidation reactions that harm cells and cause malady. The primary capacity of antioxidants is to avoid oxidation in different ways. It has been known for some time a while that antioxidants performance a very important biological role in the body by avoiding against oxidative damage(particularly oxidative DNA damage), therefore avoiding cardiovascular, neurological and carcinogenic infections and deferring chronic health problems such as cataracts**[15-17]**. Oxygen is the element fundamental for life .When cells utilize oxygen to produce energy, free radicals are produced as a result of ATP [adenosine triphosphate] generation via the mitochondria.

These by-products are mostly reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that outcome from a cellular redox process. These species play the double role like both toxic and beneficial compounds. The subtle equilibrium between their two antagonistic effects is obviously a vital aspect of life. At low or moderate levels, ROS and RNS apply helpful impacts on cellular responses and immune function. At high concentrations, they create oxidative stress, the pernicious process that may damage all cell structures**[6-10]**. Oxidative stress acting the main part in a development of chronic and progressive diseases like cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative

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diseases. The human body has numerous mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in-situ*, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by ROS and RNS, and consequently can enhance the immune defense and lower the risk of cancer and degenerative diseases[11-13,10]. The problems of harmful side effects of many antibiotics and an urgent need to therapy infectious diseases induce scientists to search for different antibacterial medications resulting from plants[18,19]. The antibacterial activities of n-butanol extract that extraction from *Conocarpus erectus* L. leaves against four selected bacterial isolate were investigated in bacteria laboratory, biology department in college of education at university of Thi qar. This extract was described to compare favorably with the standard antibiotics, Ciproda.

2. Materials and Methods

2.1. Collection and Preparation of the Plant Materials

The leaves of the plant under investigation were collected from some farms in Nassiriah city at Iraq. The plant was identified in chemistry department - College of education at University of Thi qar in Iraq. The leaves was collected and shade dried then finely powdered by electric mill, and kept in Dark glass containers and become ready for extraction process.

2.2. Preparation of Defatted Methanol Extracts

The leaves powder (300g) was drenched in 1500 ml methanol for one week at room temperature by shaking day by day followed by filtration and again extraction for four times. The organic solvents were expelled in vacuo utilizing rotatory evaporator giving known weight of crude methanol extract. The methanolic crude extract was defatted via washing sometimes with petroleum ether (60-80°C). The defatted crude methanol extract was prepared for bioassay. 20gm of a defatted methanol extract was dissolved in 80 ml distilled water, then consecutively separated with chloroform, ethyl acetate and finally with *n*-butanol (3 x 100 ml solvent) giving known weight of each respective fractions.

2.3. Primary Qualitative Analysis

Butanol extract was experienced the number of different tests such as:

Saponin test was carried out by using (5%) mercuric chloride[24].
Alkaloids test was done sing wagner's reagent[24].
Tannins was achieved by using (1%) lead acetate[29].
Carbohydrates test was done sing molish's reagent[24].
Glycosides test was carried o t sing benedict's reagent[24].
Flavonoids test was achieved by using (5N) alcoholic potassium hydroxide[30].
Phenolic compounds test was carry out by using (1%) ferric chloride[31].
Triterpinoids test was achieved by using concentration sulfuric acid[24].
Triterpines and Setrols test was achieved by using Liebermann – Burchard reagent[24].

2.4. Biological Studies

Crude defatted n-n-butanol fraction obtained from methanolic extract of the *Conocarpus erectus* L. leaves under investigation were *in-vitro* tested for their antioxidant and antimicrobial activities.

2.5. Antioxidant Activities

It is important to select and employ constant and fast methods to assay antioxidant activities, because the determination of several samples is time-consuming. In this study, two different chemical methods were utilized for the estimation of antioxidant activity of crude n-butanol fraction; 1, 1-diphenyl picrylhydrazyl scavenging activity for valuation the free radical scavenging properties, and reducing power assay utilized for dimension the total antioxidant capacity.

2.6. Scavenging ability towards 1, 1-Diphenyl Picrylhydrazyl (DPPH) Radical

The (DPPH) assay relies on the lessening of purple DPPH radicals to a yellow coloured diphenylpicrylhydrazine and the rest DPPH radicals which demonstrate maximum absorption at 517nm will be measured[35]. Two different concentrations of each sample were added to 2ml solution of 0.1 mM DPPH. An equivalent amount of methanol and DPPH attended as control. After 20 min of incubation at 37°C in the dark, an absorbance was recorded at 517 nm. The investigation was achieved in triplicates. The DPPH radical scavenging activity was calculated by the following equation: % DPPH radical scavenging activity = 1 - [*A* sample /*A* control] x 100, where A sample and A control are an absorbance of, sample and control. The decrease the absorbance of DPPH solution shows an expansion of the DPPH radical scavenging activity.

2.7. Reducing Power Assay

2 ml of every sample, ascorbic acid and butyl hydroxytoluene BHT in methanol 200 µg/ml were mixed with 2 ml of sodium phosphate buffer 0.2M, pH 6.6 and 2 ml of 1% $K_3Fe(CN)_6$ were incubated at 50°C for 20min. After adding 2 ml of trichloro acetic acid, the mixture was centrifuged at 3000 rpm for 10min. The supernatant solution 2 ml was taken out and directly mixed with 2 ml of methanol and 0.5 ml of 0.1 % ferric chloride. After incubation for 10 min, an absorbance against blank was resolved at 700 nm. Three duplicates were made for every tested sample, ascorbic acid and BHT. The expansion in absorbance of the reaction mixture shows an expanded reduction power. The lessening power activity was communicated as the number of equivalents of ascorbic acid or BHT[36].

2.8. Antibacterial assays

Antibacterial activity of n-butanol extract was determined utilizing disk diffusion, method of Mbata *et al.* with many minor modifications[32]. Stock solution was prepared via

dissolving 20 mg of an extract in sufficient amount of (DMSO) to make a final volume equal to 1 ml. 20 µl of this stock solution was infused to sterile paper disks (6 mm diameter) and dried. Mueller Hinton Agar MHA media was prepared and hardened in sterile Petri dishes. The surfaces of

the agar in all plates were swabbed with a different bacterial strain cultured in nutrient broth. The extract laden disks were placed on the surface of the swabbed agar media, and the diameter of a zone of inhibition was measured after 24 hrs of incubation at 37 ^{o}C.

2.9. Statistical Analysis

Every determination for bioassay was done triplicates and the values in table's are mean ± standard deviation .The statistical analyses were done utilizing SPSS 13.0 and Microsoft Excel program.

3. Result and Discussion

The tree of *Conocarpus erectus* L. is extensively spread in Iraq and is evergreen with a lot of flowers and fruits. Because of this and furthermore a lack of phytochemical and biological work of this plant[22,23], the authors encouraged to explore the diverse parts of *Conocarpus erectus* L. as antioxidant, anticancer and antibacterial agents. As well-known the common strategy of natural products, medication discovery from natural resources (plant, microbes, fungi, etc) began with preparatory bioassay screening (antioxidant, cytotoxic, antibacterial etc) of its crude extracts followed by bioassay-guided fractionation, segregation and structure clarification of a bioactive compounds[33]. In the current study, the n-butanol extract was isolated from *Conocarpus erectus* L. leaves bio assayed for their antioxidant properties, followed via consecutive fractionation of a defatted methanolic extract with some organic solvents of different polarities. This fraction was bio assayed as antioxidant agent utilizing two *in vitro* methods and as antibacterial toward four types of bacterial stains[25].

The chemical qualitative analysis tests results are presented in table 1 which show the presence of saponins, glycoside and carbohydrate but the polyphenols, flavonoids, tannins, alkaloids, teripinoids, terpenes and sterols gave a negative test, these ensure that butanol fraction is contents a saponins. From table 1, it was found that glycosides and carbohydrates because polyphenols is found normally connected with sacharids units. Different studies proved the presence of saponin in various medicinal plants including *Conocarpus erectus* L. Infectious diseases produced by bacteria, viruses, fungi and parasites are still a main threat to public health, despite of the great progress in human medicine Bacterial and fungal pathogens have developed several defense mechanisms against antimicrobial agents, and resistance to old and newly produced treatments is on the increase[34]. The improvement of microbial perviousness to accessible antibiotics due to random selection and conceivable side effects, have led a few authors to explore the antimicrobial activity of indigenous restorative plants in some parts of the world[37-39].

Table N0. 1: Preliminary qualitative analysis tests of n-butanol extract isolated from *Conocarpus erectus* L. leaves

Reagent	Test result	Chemical Notes	Conclusions
$FeCl_3$(1%)	-	Formation of bluish green colour	Phenols are absent
Alchohol KOH (5N)	-	No yellow precipitate	Flavonoids are absent
Wagner	-	No reddish brown precipitate	Alkaloids are absent
$Pb(Ac)_2$	-	No light brown precipitate	Tannins are absent
Molish	+	Formation of violate ring	Carbohydrate are present
Benedict	+	Formation of red precipitate	Glycosides are present
$HgCl_2$	+	Formation of white precipitate	Saponins are absent
Conc. H_2SO_4	-	No purple red color	Terpinoids are absent
Liebermann – Burchard	-	No green colour	Terpenes and sterols are absent

3.1. Antioxidant activities

3.1.1. Antioxidant effects of n-butanol fraction from the *Conocarpus* L. leaves DPPH radical scavenging ability

DPPH radical is a stable free radical that indications the maximum absorption at 517 nm, and it's broadly used to appraise the free radical scavanging ability of natural compounds. In the DPPH test, the antioxidants were able to decrease the stable radical DPPH to a yellow coloured (diphenylpicrylhydrazine). So, the antioxidant activities of the sample may be expressed such as its ability in scavanging the DPPH radical. In DPPH test, the antioxidant effect was possible to be due to a hydrogen donating ability of the extract[40]. The results in (Figure 1) indicated that the n-butanol extract from the *Conocarpus erectus* L. leaves has successful radical-scavanging activity against DPPH induced radicals. The activity of the n-butanol extract was dose dependent and portrayed by expanding scavanging activity with an ascent in sample concentrations. It was most astounding and near to the percentage inhibition of Trolox at 3mg/mlb concentration[41].

The result of the DPPH scavenging activity of *Conocarpus erectus* L. leaves n-butanol extract. It was observed that *Conocarpus erectus* L. leaves n-butanol extract inhibited DPPH free radical scavenging activity increased with increased of concentration compared with Ascorbic acid and BHT , DPPH free radical scavenging activity, of the n-butanol extract was better in higher concentration, that is, 500 µg/ml, with inhibition of 79.33% which were significantly higher than the BHT positive control (66.66%), while DPPH radical scavenging ability of the n-butanol extract was less than that of ascorbic acid (80.76%) at the same concentration (Figure 1), the result indicated that *Conocarpus erectus* L. leaves n-butanol extract has the proton-donating ability and could attend as free radical inhibitors or scavenger , acting possibly like primary antioxidant[41]. The ability of n-butanol extract to scavenge DPPH radicals, in leaves of *Conocarpus erectus* L. in our study, correlate with the results of Benhammou *et al.*, who showed antioxidant activity of n-butanol extract from the stems and root of Anabasis articulata plant using the DPPH method[42]. In an another study, Heng *et al*. reported that n-butanol extract of the bark of *Chamaecyparis* L. (A. Murray) Parl found in northern California in the USA, The n-butanol extract showed the high DPPH free radical scavenging activity, which indicated it was the most effective against DPPH• compared with ascorbic acid and BHT[43].

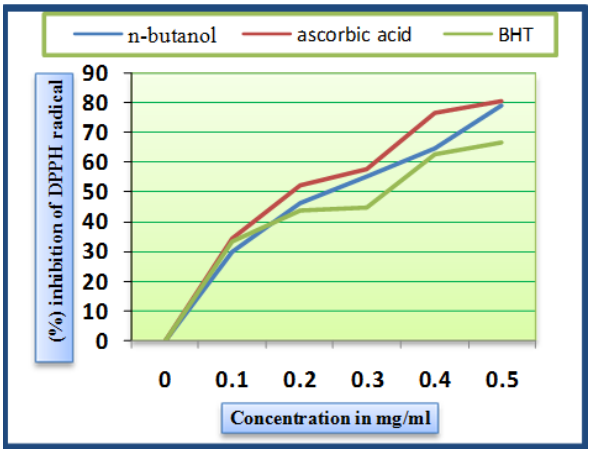

Figure No. 1: Scavenging effects of n-butanol extract isolated from *Conocarpus erectus* L. leaves on DPPH radical. The results are means ± SD (n=5).

3.1.2. Ferric reducing ability

Figure no. 2 presents a ferric reducing ability of n-butanol extract. Our results indicate that n-butanol extract exhibited the highest FRAP value, in the all concentrations, compared with ascorbic acid and BHT, n-butanol extract had the highest ferric reducing activity (1.55nm) compared with a positive controls, ascorbic acid (0.9nm) and BHT (0.45nm), in concentration of 300 µg/ml for all samples. The significantly higher absorbance values of n-butanol extract than ascorbic acid and BHT at lower concentrations (100 µg/ml) suggests that the n-butanol extract especially at such concentrations, has high redox potentials and can acts as reducing agent, hydrogen donor and singlet oxygen quencher[44]. This observation is similar to that reported for *A. longicaulis*[45]. Ferric reducing, in this study, ferric reducing ability of n-butanol extract correlate with the results of the reducing capacity of the test compound assists as the indicator of its potential antioxidant activity due to a presence of reductants[46]. The reductive ability of n-butanol extract from the leaves of *Conocarpus erectus* L. in the dose reliant on formation of Perl's Prussian blue to reduce ferric ions to its ferrous form (Figure 2). The result shown that n-butanol extract of *Conocarpus erectus* L. leaves is the electron donor that can terminate radical chain reaction[40].

Antioxidants may be reductant and inactivation of oxidants by means of reductants can be portrayed as redox reactions in which one reaction species is reduced at an expense of an oxidation of the other[44]. The attendance of reductants, like antioxidant substances in a sample, causes a reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The reducing power ability of an extract increased with increasing of concentration which proposes that the electron donating ability of the extract is concentration dependent.

Some *in-vitro* methods have been produced to evaluate the total antioxidant capacity of fruits, vegetables, and beverages. One of these, is the (FRAP) assay. This is a colorimetric method based on the diminishment of a ferric tripyridy ltriazine (TPTZ) complex to its ferrous form. This evaluate

creates a deep blue complex in an absorption maximum at (593 nm)[47]. The FRAP analyze is a simple, suitable and reproducible technique that was initially created to quantify the plasma antioxidant capacity, but is presently generally utilized in the antioxidant studies of other biological samples, for example food, plant extracts, juices and drinks, and so forth[48]. The current experimental procedure has recognized that break of 4 min and a temperature incubation of 37 º would be appropriate conditions to test the total antioxidant capacity, of most samples, because the redox reactions continue so quickly that all reactions are finished. However, the application of like experimental conditions, to samples of dietary antioxidants, fruits, vegetables, and drinks, is not appropriate to evaluate their total Fe-TPTZ-reducing capacity. This infers that the FRAP values can vary extremely depending on a time scale of analysis[48-50].

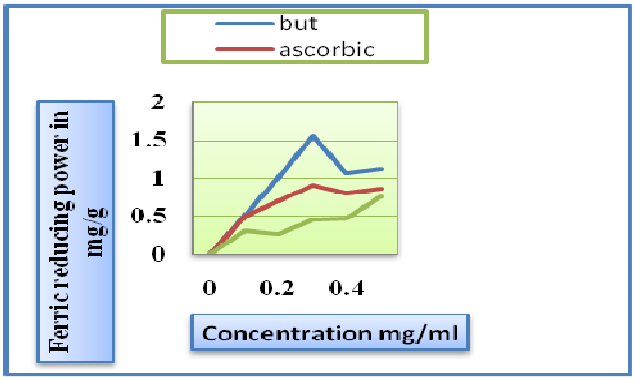

Figure No. 2: Ferric reducing ability of n-butanol DPPH radical. The results are means ± SD (n=5) extract isolated from *Conocarpus erectus* L. Leaves on DPPH radical. The results are means ± SD (n=5)

3.2. Antibacterial activity of extract

The results of antibacterial activities for n-butanol extract are given in table 2. The n-butanol extract of leaves of *Conocarpus erectus* L. was tested against 4 bacterial strains. Against any microbial species, the activity of this extract was measured to be present if a diameter of the inhibition zone was equivalent to or greater than 7 mm. The antibacterial activity was found to be highest for *Streptococcus pneumoniae* where the zone of inhibition was noted 22mm. The clinical isolate of *Escherichia coli* was also found to be sensitive against this extract showing the zone of 21mm which is an agreement with the study of Akinpelu *et al*.[17]. Less activity (20mm) was found in case of *Staphylococcus aureus* and (19mm) *Enterobacter*. The positive control (Ciprodar) showed maximum activity against *Straptococcus* (25mm) and against *Staph. aureus* (23mm). This results appearance the presence of high antibacterial activities in the n-butanol extract of *Conocarpus erectus* L. leaves thus indicating plant to be of medicinal value.

The reason of a differential sensitivity between gram-positive form and gram-negative bacterial strains couldn't be attributed to their morphological contrasts or offered to their chemical structures. Gram negative bacteria have the external phospholipids membrane[51]. The cell wall of gram-positive bacteria is less chemically complex than that of the gram negative bacteria[52]. Gram-negative bacteria is recognized to be resistant to an action of numerous antimicrobial agents, including antimicrobial plant extracts [53,54].

Table No. 2: Antibacterial activity of n-butanol extract of *Conocarpus erectus* L. on various clinical strains

Organisms	Bacterial stains code	Gram Stain	N-butanol extract	Control (Ciprodar)
Staph.aureus	(ATCC-29737)	+	20	23
Straptococcus	(ATCC -49619)	+	21	25
E.coli	(ATCC-14169)	-	22	18
Enterobacter	(ATCC®-23355™)	-	19	19
DMSO			0	

4. Conclusion

This study confirmed that *Conocarpus erectus* L. leaves n-butanol extract could be utilized to preclude damage produced via free radicals and infections produced via pathogenic bacteria, this extract had a highest antioxidant capacity, The antioxidant mechanisms of n-butanol extract may be due to a strong hydrogen donating capability of an active compounds contained in the sample, which may reduce a concentrations of DPPH• and FRAP• free radicals. Studies are in advance to isolate and identify a chemical compounds that add to the total anti-radical activities and to better understand their mechanism of action as antioxidants, and the present study proved the good medicinal activities of n-butanol extract of *Conocarpus erectus* L. leaves against growth of some bacteria represented by *Staph. Aureus*, *Straptococcus* (positive to Gram stain), *E.coli*, *Enterobacter* (negative to Gram stain).

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5. References

[1]. Redha A., Al-Mansour N., Suleman P., Afzal M., and Al-Hasan R., Leaf traits and histochemistry of trichomes of *Conocarpus lancifolius* a *Combretaceae* in Semi-Arid Conditions, American Journal of Plant Science 2011;2,165-174.

[2]. Saad TM., Farheen A., Nureen Z., Zeeshan M., Maria F., Ayesha J., Antibacterial and Antifungal Activity of *Conocarpus Lancifolius* engl. (*Combretaceae*), J App Pharm. 2014;6:2:153-155.

[3]. Saroya AS., Herbal Drug Industry, In: Saroya AS, Ed. Herbalism, Phytochemistry and Ethnopharmacology. Enfield, USA: Science Publishers;2011; p. 17.

[4]. Daniela D., Marina D., Irene S., Mirela B., Monica R., Kamal D and Terezinha D., The Microbial Quality Aspects and Decontamination Approaches for the Herbal Medicinal Plants and Products: An in-Depth Review, Current Pharmaceutical Design 2016;22:27:4264–4287.

[5]. WHO, WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues 2007; p. 213.

[6]. Halliwell B., Gutteridge JMC., Free radicals in biology and medicine, 4th. Oxford, UK: Clarendon Press; 2007.

[7]. Bahorun T., Soobrattee MA., Luximon-Ramma V., Aruoma OI., Free radicals and antioxidants in cardiovascular health and disease, Internet J. Med. Update 2006;1:2:1–17.

[8]. Pacher P., Beckman JS., Liaudet L., Nitric oxide and peroxynitrite in health and disease, Physiol. Rev. 2007;87:1:315–424.

[9]. Genestra M., Oxyl radicals, redox-sensitive signalling cascades and antioxidants, Review, Cell Signal, 2007;19:9:1807–1819.

[10]. Lien A., Hua H., and Chuong P., Free Radicals, Antioxidants in Disease and Health; Int J Biomed Sci. 2008;4:2:89–96.

[11]. Valko M., Rhodes CJ., Moncol J., Izakovic M., Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chem. Biol. Interact. 2006;160:1–40.

[12]. Valko M., Morris H., Cronin MTD., Metals, toxicity and oxidative stress, Curr. Med. Chem. 2005;12:1161–1208.

[13]. Chatterjee M., Saluja R., Kanneganti S., Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats, Cell Mol. Biol. 2007;53:1:84–93.

[14]. Bugno A., Buzzo A., Nakamura C., Pereira T., Matos D., Pinto T., Avaliaçao da contaminaçao microbiana em drogas vegetais, Brazilian J Pharm Sci 2005;41:4:491-7.

[15]. Williamson G., Day AJ., Plumb GW., Couteau D., Human metabolic pathways of dietary flavonoids and cinnamates, Biochem. Soc. Trans. 2000;28:16-22.

[16]. Morton LW., Cacceta RA., Puddey IB., Croft KD., Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular Disease, Clin. Exp. Pharmacol. Physiol. 2000;27:3:152-159.

[17]. Akinpelu B., Igbeneghu O., Awotunde A., Iwalewa E and Oyedapo O., Antioxidant and antibacterial activities of saponin fractions of *Erythropheleum suaveolens* (Guill. and Perri.) stem bark extract, Academic Journals 2014;9:18:826-833

[18]. Aliero A., Aliero B., and Buhari U., Preliminary phytochemical and antibacterial screening of Scadoxus multiflorus, Int. Jor. P. App. Scs. 2008; 2:4:13-17.

[19]. Resmi M., Linar Z., Muchtaridi., Supriyatna., Identification and Antibacterial Activity of Methanol Extract of *Luffa Acutangula* Roxb, MHSJ 2012;12:70-77.

[20]. Baroon Z., Razzaque MA., Nutritional evaluation and palatability trial of ensiled *Conocarpus Greenery*

residues, Exper Agric. 2012;48:1:138-147.

[21]. Malik S., Bashir A., Muhammad U and Khurram A., Antidiabetic potential of *Conocarpus lancifolius*, Bangladesh J Pharmacol. 2014;9:2:244-249.

[22]. Nahla AA., A trimethoxyellagic acid glucuronide from *Conocarpus erectus* leaves: Isolation, characterization and assay of antioxidant capacity, Pharm. Biol. 2010; 48:3: 328–332.

[23]. Jagessar RC and Cox M., Phytochemical Screening of the $CHCl_3$ and CH_3CH_2OH Extract of Stems, Twigs, Roots and Barks of *Conocarpus erectus* L. International Journal of Academic Research 2010;2:36-45.

[24]. Aisha H., Amina R., Patrice S., Redha A., The Allelopathic Potential of *Conocarpus lancifolius* (Engl.) Leaves on Dicot (*Vigna sinensis* L.), Monocot (*Zea mays* L.) and Soil-Borne Pathogenic Fungi, American Journal of Plant Sciences, 2014;5:19:2889-2903.

[25]. El-Sayed S., Salih A., Mohamed M., Mortada M and Eman A., Phytochemical Studies and Evaluation of Antioxidant, Anticancer and Antimicrobial Properties of *Conocarpus erectus* L., Growing in Taif, Saudi Arabia 9. 2012;2:2:93-112.

[26]. Abdel-Hameed ES., Bazaid SA., Shohayeb MM., El-Sayed MM and El-Wakil EA., Phytochemical Studies and Evaluation of Antioxidant, Anticancer and Antimicrobial Properties of *Conocarpus erectus* L., Growing in Taif, European J Medic Plan. 2011;2:93-112.

[27]. Abdel-Hameed ES., Abdel SAB. and Sabra NA., Protective Effect of *Conocarpus erectus* Extracts on CCl 4-Induced Chronic Liver Injury in Mice, Global J Pharm 2013;7:1:52-60.

[28]. Harborne JB,. Photochemical methods, 2nd ed. Chapman and Hall, New York, USA 1984.

[29]. Molan A., Mcnebb W., Attowd G., Min B., Peters J. and Barry T., The effect of condensed tannins from lotus species protein degradation and bacterial growth in the rumen, J.Nat.Pro.Soc. 1997;22:246.

[30]. Al-Assadi I., Study of hypoglycemia and antihyperglycemia action of *Olea europae* in animals and human, Ph.D. thesis, college of science, Basrah University, Iraq. 2001.

[31]. Waterman P. and Mole S., Analysis of phenolic plant metabolites, J. Plant Medica. 1994;55:1.

[32]. Mbata TI., Debiao LU., and Saikia A., Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on Listeria monocytogenes, African Journal of Biotechnology 2008;7:10:1571-1573.

[33]. Abdel-Hameed ES., Total phenolic contents and free radicals scavenging activity of certain Egyptian *Ficus species* leaf samples, Food Chem. 2009;114:4:1271–1277.

[34]. Buvaneswari S., Raadha CK., Krishnaveni N., Jayashree S., *In-vitro* Antimicrobial activity of *Psidium guajava* against clinically important strains, E-Journal of Life Sci. 2011;1-14.

[35]. Shirwaikar A., Prabhu K. and Punitha ISR., *In-vitro* antioxidant studies of *Sphaeranthus indicus*, Indian Journal of Experimental Biology 2006;44:993-998.

[36]. Oyaizu M., Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucosamine, J. Nutr. 1986;44:307-315.

[37]. Zampini IC., Cuello S., Alberto MR., Almeida RD., Solorzano E., Isla E., Antimicrobial activity of selected plant species from the *Argentine puna* against sensitive and multi-resistant bacteria, J. Ethnopharm. 2009;124: 499.

[38]. Abdel-Hameed ES., El-Nahas HA., Abo-Sedera SA., Antischistosomal and antimicrobial activities of some Egyptian plant species, Pharm. Biol. 2008;46:9:626-633.

[39]. Barbour EK., Al-Sharif M., Sagherian VT., Habre AN., Talhouk RS., Talhouk SN., Screening of selected indigenous plants of Lebanon for antimicrobial activity, J. Ethnopharm. 2004;93:1:1-7.

[40]. Conforti F., Loizzo MR., Statti GA and Menichini F., Comparative radical scavenging and anti diabetic activities of methanolic extract and fractions from *Achillea ligustica*, All Biol Pharm Bull. 2005;28:9: 1791-1794.

[41]. Alli Y and Adanlawo I., *In-vitro* and *In-vivo* antioxidant activity of saponin extracted from the root of *Garcinia kola* (bitter kola) on alloxan-induced diabetic rats, WJPPS 2014;3:7:2278–4357 .

[42]. Nsimba RY., Kikuzaki H., Konishi Y., Antioxidant activity of various extract fractions of *Chenopodium quinoa* and Amaraathus sp. Seeds, Food Chem. 2008;106:760-766.

[43]. Benhammou N., Ghambaza N, Benabdelkader S, Atik-Bekkara F and Kadifkova T. Phytochemicals and antioxidant properties of extracts from the root and stems of Anabasis articulate.2013; 20(5): 2057-2063.

[44]. Heng G, Todd F., Chung Y., Hse and Thomas L., Antioxidant activity of extracts from the bark of *Chamaecyparis lawsoniana* (A. Murray) Parl 2006;60: 459–462.

[45]. Tuba A. and Gülçin I., Antioxidant and radical scavenging properties of curcumin, Chem. Biol. Interact. 2008;174:1:27-37.

[46]. Zubia M., Robledo D., Freile-Pelegrin Y., Antioxidant activities in tropical marine macroalgae from the *Yucatan Peninsula*, Mexico, J Appl Phycol 2007;19:5: 449-458.

[47]. Karmakar I., Dolai N., Saha P., Sarkar N., Bala A., Haldar PK., Scavenging activity of *Curcuma caesia* rhizome against reactive oxygen and nitrogen species. Orient Pharm Exp Med. 2011;11:221-228.

[48]. Benzie I. and Strain J., The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay, Analytical Biochemistry 1996;239:1:70-76.

[49]. Pulido R., Bravo L. and F., Saura-Calixto., Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay, Journal of Agricultural and Food Chemistry 2000; 48:8:3396-3402.

[50]. Imeh U. and Khokhar S., Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations, Journal of Agricultural and Food Chemistry 2002;50:22:6301-6306.

[51]. Henríquez C., Carrasco C., Gómez M., Brunser O. and Speisky H., Slow and fast-reacting antioxidants from berries: Their evaluation through the FRAP (Ferric Reducing Antioxidant Power) assay, Acta Horticulturae 2008; 777:82::531-536.

[52]. Hugo WB., Bacteria. In: Pharmaceutical microbiology, Hugo WB. and Russell AD.. Oxford, Blackwell Scientifc Publications 1998.

[53]. Lamikanra A., Essential Microbiology for students and practitioner of Pharmacy, Medicine and microbiology, 2nd ed. Amkra books 2010.

[54]. Kambezi L., Afolayan AJ., Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhea*, Afr. J. Biotechnol. 2008;7:1:012-015.

[55]. El-Mahmood AM., Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections, J. Med. Plants Res. 2009;3:7:498-505.

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