Antioxidant and analgesic activities of leaf stem and root essential oils of *Corchorus olitorius* L. (Tiliaceae) from Nigeria

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Original submitted in on 22nd January 2018. Published online at www.m.elewa.org on 31st March 2018
https://dx.doi.org/10.4314/jab.v123i1.8

ABSTRACT

Objectives: *Corchorus olitorius* (Linn.) (Tiliaceae) (Jute is an edible fibre crop found in Asia and Africa. It has medicinal value and is very nutritious. This research was designed to investigate the antioxidant and analgesic properties of the essential oils of *Corchorus olitorius* in the leaf, stem, root, fruit and flower.

Methodology and Results: *Corchorus olitorius* plant was collected from cultivated farmlands in Ibadan, Nigeria. Essential oils from parts of plant were extracted by hydro-distillation, using all glass Clevenger apparatus. The oils were analysed using GC-MS. Its antioxidant activity was determined by measuring the decrease in the visible absorbance of 2, 2, diphenyl-1-picrylhydrazyl (DPPH) on addition of the plant essential oils and compared with synthetic and natural standards such as vitamin C, garlic, bitter kola, ginger and carrots. Analgesic property of essential oil was investigated by measuring the number of acetic acid induced writhing in mice. IC50 values were as follows; leaf; 82.0 µg/ml, stem; 51.0 µg/ml and root essential oils; 49.0µg/ml, vitamin C; 33.0 µg/ml, garlic; 49.0µg/ml, bitter kola; 31.0 µg/ml, ginger; 31.0 µg/ml and carrots; 31.0 µg/ml. The best analgesic activities were recorded for root (100%) on 15 ml/kg; stem (93.67%) on 5 ml/kg; leaf (79.27%) on 5 ml/kg and root essential oil (78.69%) on 5 ml/kg bodyweight of mice.

Conclusions and Application of findings: This shows that root has highest antioxidant activity and is comparable to garlic. The compounds present are suggested to be responsible for antioxidant and analgesic activities of the essential oils of *Corchorus olitorius* either synergistically or individually, which agrees with ethno medicinal claims on the plant.

Keywords: *Corchorus olitorius*, essential oil, antioxidant, analgesic activity, 2, 2 diphenyl-1-picrylhydrazyl, Diclofenac sodium.

INTRODUCTION

Natural products are chemical compounds or secondary metabolites found in plants, microbes and animals (Desaubry *et al*., 2014). Plant parts with their phytochemical constituents are good sources of essential oils (Sadgrove & Jones, 2015). Essential oils are complex mixtures of volatile lipophilic organic constituents sourced from all parts of plants and widely in bryophytes such as liverworts. (Asakawa *et al*., 2012). Each of these constituents contributes to the beneficial or adverse effects of these oils (Buchbauer, 2000). Essential oils are important natural products used variously in fields such as
perfumery, cosmetology, aromatherapy, phytotherapy, food flavour and nutrition (Buchbauer, 2000). An essential oil with therapeutic value can enhance its use in perfumery. Aromatherapy refers to the use of fragrances or volatiles for therapeutic and/or prophylactic purposes. The beneficial or toxic effect of these essential oils can be attributed directly to the component compounds. There is then a need for concise knowledge of essential oil compositions so as to tap any beneficial value or prevent any adverse effect from occurring (Buchbauer, 2000). Adequate information on essential oil compositions helps in its proper use and prevents abuse. This information can best be obtained by use of capillary – GC experiments (Buchbauer, 2000). Differences have been noted in activity of whole essential oils and their main components on different biological models studied (Lahlou, 2002, 2003; Lahlou & Berrada, 2003; Lahlou & Berrada, 2001; Lahlou et al., 2001; Lahlou et al., 2000). The activity of essential oils was found to be less than that of major constituents such as phenolic, terpenic, ketonic and alcoholic (Lahlou, 2004). Further studies relating composition and activity suggest that biological activity of essential oils may be attributed to their major (phenolic, terpenic, ketonic or alcoholic) compounds and minor (alkanes, alkenes or alkynes) components present in these oils (Lahlou, 2004). Thus, it is possible that essential oil bioactivity is due to synergy and not individual component activity. Some reported biological activities of essential oil include antimicrobial activity (Remmal et al., 1993; Morris et al., 1978; Santos et al., 1998; Essien et al., 2012), liceidal and niticidal activity (Lahlou et al., 2000; Lahlou & Berrada, 2003), anti-inflammatory and analgesic activity (Santos et al., 1998), antiproliferative activity (Essien et al., 2012). Phytochemical components in plants have been shown to be responsible for bioactivities. These phytochemicals impart physiological activities and may offer a variety of health benefits such as antioxidant, antibacterial, anti-inflammatory or anticancer activity (Anupam et al., 2008). Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. They are substances that neutralize free radicals or their action. Antioxidants are found in many foods, including fruits and vegetables. A free radical inhibitor is a specie that inhibits a free radical reaction. Such inhibitors usually act by undergoing reactions with reactive free radicals to form relatively stable non-reactive free radicals (Fessenden & Fessenden, 1986). Free radical inhibitors that exist in food or are used in addition to foods are known as antioxidants or preservatives. Typical examples of these are vitamin E, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and vitamin C. Corchorus olitorius that belongs to the family Tiliaceae (Linden) is a fibre crop. It is an annual herb with leaves and roots applied in herbal medicine and eaten as vegetable by local people in Nigeria (Oyedele et al., 2006), Africa (Velempini et al., 2003), East Malaysia, India, and Philippine (Zeghichi et al., 2003). Mbaye et al (2001) reported that the genus contains forty species throughout the tropics. Edwards (1991) stated that thirty species are found in Africa and few species in Nigeria. The species found in Nigeria include Corchorus olitorius, Corchorus tridens, Corchorus aestans and Corchorus fascicularis. These species are distributed in the country and are popularly called Ewedu in Yoruba, which is the south western region. Atiever or Adigbor in Tiv, Epo-Ipin (Mbo) in Idoma, Anzile in Etulo, Ayoyo in Gbagi and Lalo in Hausa (Agishi, 2010). The leaves are rich in beta-carotene, iron, calcium and vitamin C. The plant has significant antioxidant activity compared to vitamin E (tocopherol) (Ayodele, 2005). The seeds are used as purgative and have been found to contain cardenolide on preliminary analysis (Gupta et al., 2003). Methanol extracts of the seeds have been reported to possess a broad spectrum of antibacterial activity (Palli et al., 2006). Leaves have been found to suppress elevation of post prandial blood glucose levels in rats and humans (Innami et al., 2005). The objectives of the research work are:

- Extraction of essential oils from leaf, stem, root, fruit and flower of Corchorus olitorius.
- Gas chromatography-Mass spectrometry (GC-MS) analyses of the extracted essential oils to determine their chemical composition.
- Investigation of the antioxidant properties of the leaf, stem and root essential oils of Corchorus olitorius using 2, 2-diphenyl-1-picrylhydrazyl (DPPH).
Corchorus olitorius.\)

**MATERIALS AND METHODS**

**Collection of Plant Materials: Corchorus olitorius** plant was collected from cultivated farmlands in Ibadan, Oyo state. The plant was identified and authenticated in the herbarium, Department of Botany, University of Ibadan, Ibadan, where a specimen was deposited and a voucher was issued (UIH 22526). The plant was collected in the morning after which it was separated into leaf, stem, root, fruit and flower parts.

**Preparation of Plant Material:** The freshly obtained plant material was washed with water to exclude contaminants. Each part of the plant; leaf, stem, root, fruit and flower was separated and weighed.

**Drugs and Chemicals:** Diclofenac sodium was purchased from Normed Pharmacy Ltd, a registered pharmacy (Gboko, Nigeria). DPPH and all other chemicals were purchased from Sigma-Aldrich (Germany).

**Experimental Animals:** Albino mice of both sexes weighing between 24 to 32 g obtained from the animal house of Benue State University, Makurdi were used for the animal study. Animals were acclimatized at room temperature (29°C) with a relative humidity of 70% in standard cages for 4 to 5 days prior to commencement of the experiment. Throughout the period of study, animals were kept under hygienic conditions by constant cleaning and removal of faeces and spilled feeds from cages daily. The study was approved by the Animal Welfare and Ethics Committee of University of Mkur, Mkur, Benue State, Nigeria. All conditions of animals used were as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals.

**Extraction and Identification of the Essential Oil Components Using GC-MS:** Essential oil extractions were carried out by the use of an all-glass Clevenger-type apparatus designed to British Pharmacopoeia specifications. 250 g each of leaf stem and roots, 150 g of fruit and 120 g of flower of Corchorus olitorius were crushed and hydro-distilled for 2 hours. 2.0 ml each of distilled n-hexane were used to remove the essential oils. The oils were then stored in vials and refrigerated. Essential oil composition of leaves of Corchorus olitorius was determined by Gas Chromatography-Mass Spectrometry (GC-MS) using an Agilent 7890A Gas Chromatograph hyphenated with an Agilent mass detector triple Quad 7000A in EI mode at 70Ev (m/z range 40-600 amu) with an ion source temperature of 250°C and an Agilent ChemStation data system. GC column was equipped with an HP-5MS column (30 m x 250 µm x 0.25 µm) a split-split less injector heated at 200°C and a flame ionization Detector (FID) at 230°C. Oven temperature was programmed to be: Initial temperature 40°C for 5min, increased 5°C/min to 180°C for 6 min and then 10°C/min to 280°C for 12 min. Carrier gas was Helium at flow rate of 1 mL/min. Injection volume was 2.0µL (split ratio 1:20). GC-MS QP 2010 Plus was used for the analyses of stem, root, fruit and flower of Corchorus olitorius. Ion source and interface temperature was 250 °C; solvent cut time 2.5 minutes with relative detector gain mode and threshold 3000; scan MS ACQ mode; detector FID; mass range of m/z 40-400. Identification of the essential oil components was based on their Kovats indices (Alencar et al., 1984) and retention indices (determined with reference to homologous series of n-alkanes), along with comparison of their mass spectral fragmentation patterns by computer matching with in-built data and commercials such as Joulain & Koenig (1998), Adams (1995), and Massada (1976) Libraries. Other search libraries used include database/NIST08.L.

**Determination of Antioxidant Activities of the Leaf, Stem and Root Essential Oils of Corchorus olitorius**

**Using DPPH (2, 2-diphenyl-1-picrylhydrazyl):** The antioxidant activity of each essential oil was measured in terms of free radical scavenging activity using the stable radical DPPH (Brand-Williams et al., 1995). To 1 ml of essential oil each of the concentrations; (31.25, 62.5, 125.0, 250.0 and 500.0 µg/ml), 0.5ml of 1mM DPPH in hexane was added. A blank solution was then prepared containing 1ml of hexane and 0.5ml of 1mM DPPH. The experiments were incubated for 15 mins. Hexane was used in zeroing the UV spectrophotometer and absorbance was obtained at wavelength of 517 nm. Radical scavenging activity was calculated using the following formula (Miliauskas et al., 2004).

\[
\%\text{Inhibition of DPPH} = \left(\frac{A_B - A_A}{A_B}\right) \times 100
\]

Where \(A_B\) is the absorption of blank and \(A_A\) is the absorption of essential oils. The experiment was carried out in triplicates.

**Analgesic Activity Assay**

**Acetic Acid Induced Writhing Test:** The abdominal constriction was induced in mice (weighing 15-32 g) by intraperitoneal injection of 1% (v/v) acetic acid (2.3 ml/kg), as described by Santos et al., (1998). Animals were pre-treated with leaf, stem and root essential oils of C.
olitorius at doses of 5, 10 and 15 ml/kg, intraperitoneally, 45 minutes before acetic acid administration. Control animals received 2ml volume of hexane and the positive control animals were treated with the reference analgesic drug; Diclofenac sodium (40 mg/kg bodyweight). Animals were treated one at a time. The number of abdominal constrictions was cumulatively counted over a period of 20 minutes after acetic acid administration. The percentage inhibition of analgesic activity was calculated using the following formula (Santos et al., 1998)

\[ \% \text{ Analgesic Activity} = \frac{\text{Mean writhing count (control group} - \text{treated group)}}{\text{Mean writhing count of control group}} \times 100 \]

**Statistical Analysis:** Data obtained was expressed as Mean± Standard Error of mean and analyzed using the Analysis of Variance ‘ANOVA’ (Welkowitz, 1976) and SPSS (version 20) where applicable. Values at P<0.05 were regarded as significant in comparison with appropriate controls.

**RESULTS**

**Antioxidant Activity:** Antioxidant activity is measured among other methods by the use of 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) scavenging ability. Here the free radical scavenging power of essential oils to mop up DPPH; a stable radical, is used to determine the % inhibition which on graphical representation gives the mean inhibitory concentration (IC\(_{50}\)) of the essential oil sample.

Free radical scavenging activities of leaf, stem and root essential oils of *Corchorus olitorius* determined by measuring the decrease in the visible absorbance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and compared with the synthetic and plant antioxidants such as vitamin C, garlic, *Garcinia kola*, ginger and carrots. The mean inhibitory concentration of essential oils needed to decrease the initial absorbance of DPPH by 50% was determined graphically. IC\(_{50}\) values of leaf, stem and root essential oils, ascorbic acid (vitamin C), *Allium sativum* (garlic), *Garcinia kola* (Bitter kola), *Daucus carota* (carrot) (CA) and *Zingiber officinale* (ginger) (ZO) against concentration.

**Figure 1:** % Inhibition of DPPH by leaf (LFE), stem (STE), root (RTE), *Allium sativum* (garlic) (GA), Ascorbic acid (VC), *Garcinia kola* (Bitter kola) (GK), *Daucus carota* (carrot) (CA) and *Zingiber officinale* (ginger) (ZO) against concentration.

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µg/ml, 49.0 µg/ml, 33.0 µg/ml, 49.0 µg/ml, 31.0 µg/ml, 31.0 µg/ml and 31.0 µg/ml respectively.

**Analgesic Activity**

**Acute Toxicity Study:** The Lethal Dosage (LD$_{50}$) gives a measure of the immediate or acute toxicity of a test substance (Lorke, 1983). The mice were alive and never manifested signs of toxicity at the maximum administered dosage of 20 ml/kg body weight for leaf, stem and root essential oils. There was no detectable sign of toxicity on the animals, which suggests that the essential oils are safe at this level. Since the animals had limited liquid consumptions, the test for safety of these essential oils was left at this stage.

**DISCUSSION**

IC$_{50}$ values of leaf, stem and root essential oils, Ascorbic acid (vitamin C), Allium sativum (garlic), Garcinia kola (Bitter kola), Zingiber officinale (ginger) and Daucus carota (carrots) are 82.0% v/v, 51.0% v/v, 49.0% v/v, 33.0 µg/ml, 49.0 µg/ml, 31.0 µg/ml, 31.0 µg/ml and 31.0 µg/ml respectively. This indicates good antioxidant...
activity for the root and stem essential oils. Adepoju (2009) highlighted the importance of vitamin C and carotenoids, which have been linked with nutritionally related ailments such as cancers, coronary heart diseases, obesity and diabetes mellitus as reported by Mc Dougall et al. (1996) and Larrauri et al. (1996). Since stem essential oils were more active than vitamin C, stem essential oils of *C. olitorius* can be used as a source of vitamin C. The leaf oils showed least IC$_{50}$ value hence relatively less antioxidant power. Bruice (2007) reports that unwanted radicals are removed by radical inhibitors; compounds that destroy reactive radicals by either converting them into unreactive radicals or into compounds with only paired electrons. The exact mode of scavenging of *Corchorus olitorius* plant essential oils cannot be explained presently but it can be inferred that the high presence of phenolic compounds in its root and stem essential oils explains its antioxidant activity. Preliminary studies by Zakaria et al. (2005) showed that the aqueous extract of *C. olitorius* possessed peripherally and centrally mediated antinociceptive, which were both mediated, at least in part, via the opioid receptor. This confirms the analgesic properties of the essential oils of *Corchorus olitorius* as presented in this report. Acetic acid induced abdominal constrictions are useful experimental tools in the testing of new analgesic drugs (Otterness and Bliven, 1985), this is because the intraperitoneal administration of acetic acid in mice leads to the release of endogenous substances such as serotonin, histamine as well as arachidonic acid which results in synthesis of prostaglandins via the cyclooxygenase enzyme (Davies et al., 1984). the special nerve endings that sense pain are very sensitive to prostaglandins. The analgesic activity of the leaf essential oils of *C. olitorius* follows no particular pattern. It is worthy to note that the lowest dose of 5 ml/kg bodyweight exhibits best analgesic activity in the acetic acid induced abdominal writhing in mice as compared to the 10 ml and 15 ml/kg bodyweight administration. Averagely, leaf essential oils had better inhibition (79.27 %) as compared to the standard drug, diclofenac at 26.70 % inhibition. This good activity may be because of either individual components in the oil or synergistic effect. This according to Lahlou and Berrada (2003), the biological activity of essences from the aromatic plants may be attributed to both their major components (alcoholic, terpenic, phenolic or ketonic compounds) and to the minor ones present in the compound. Lahlou (2003) suggests the possibility of a synergistic action of the constituent compounds. The stem essential oils showed good promise against pain. 5 ml/kg bodyweight stem essential oils had 93.67% analgesic activity. A pattern though not very distinct existed between the administration doses. The higher the dosage of administration, the poorer the analgesic effect (10 ml; 12.67% and 15 ml; 12.28%). The reason for this is not known yet but individual assessment of the antinociceptive effect of the major components may offer suggestions. The abundance of esters and alcohols in the stem essential oils may be a pointer to its activity. Lahlou (2003, 2002 and 2001) and Lahlou et al. (2001) reported that alcoholic, phenolic, terpenic or ketonic compounds attribute biological activity to essential oils. This then explains why stem essential oils have better analgesic activity than diclofenac and hence can be harnessed for use.

The best result is the 15 ml/kg body weight administration of root essential oils of *Corchorus olitorius*. 100% analgesic activity was recorded. This result may be due to synergism considering the almost even distribution in the percentage of component fractions of the stem oils. This result far outshines the standard drug, diclofenac that stands at 26.70%. The root essential oil of *Corchorus olitorius* if harnessed properly may be an analgesic drug for the future.

**CONCLUSION**

The compounds identified may be responsible for the bioactivities of the essential oils of *Corchorus olitorius* either synergistically or individually. The results suggest that *Corchorus olitorius* essential oils may be used for analgesic and antioxidant purposes.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest

**ACKNOWLEDGEMENT**

The authors are grateful to the Management of University of Ibadan, Ibadan and the University of Mkar, Mkar for providing facilities and funding this research.
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