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Review Article

A review of phytochemical and biological studies on *Conocarpus erectus* (Combretaceae)

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Abstract

***Conocarpus erectus* is a low branching evergreen shrub or tree with a typical height of up to 40 feet. It contains phenols such as flavonoids and tannins as major constituents. The extract of *C. erectus* from different parts (leaves, stems, fruits, and flowers) showed high antioxidant, hepatoprotective and anticancer activity due to the presence of phenolic compounds. Tannins have high antimicrobial activity than other phenolic compounds. It is an attempt to review the pharmacognostic characteristics, traditional uses, phytochemistry and biological activities of the plant.**

Keywords: Botanical, phytochemical, Biological studies, *Conocarpus erectus*

Introduction:

Historically, natural products have been used since ancient times and in folklore for the treatment of many diseases and illness. Natural product chemistry methodologies enable a vast array of bioactive secondary metabolites from terrestrial and marine sources to be discovered (Dias *et al*. 2012). Thus natural products are considered as valuable source of drugs development against various diseases (Chaudhary *et al.* 2010). According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicines which has compounds derived from medicinal plants (Nagarajan *et al.* 2013). There is an ever increasing demand to pharmacologically evaluate various plants used in Ayurvedic system of medicine (Kumar *et al.* 2011).

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The present attempt is to review and compile updated information on various aspects of *C. erectus* L. a plant used for road-side medians, parking lots, screening hedges, landscaping purposes as well as folklore medicine. The brief taxonomical data of the plant is described below:

***Conocarpus erectus* L.**

Class:	Equisetopsida C. Agardh
Subclass:	Magnoliidae Novák ex Takht.
Superorder:	Rosanae Takht.
Order:	Myrtales Juss. ex Bercht. & J. Presl
Family:	Combretaceae R. Br.
Genus:	*Conocarpus* L.

***Conocarpus erectus* L.** is commonly known as Button mangrove, Buttonwood (English), Buttobush, Mangle boton, Botocillo (Spanish), Mangle botoncillo, Mangle negro, Mangle prieto, Yana, Marequito, Zaragosa, Mangle jeli, Jele, Manglier gris, Paletuvier, Mangel, Grijze mangel, Witte mangel, Mangue, Mangue branco, Mangue de botao.

Uses: The plant is used as folk remedy in anemia, catarrh, conjunctivitis, gonorrhea, diabetes, diarrhea, fever, headache, bleeding, tumors, orchitis, pricklyheat, swellings, and syphilis (Duke and Wain, 1981; Irvine, 1961; Morton, 1981). The leaves are eaten, or their decoction drunk, for fever (Irvine, 1961). Wood is used for fenceposts, crossties, turnery, boat building, firewood and landscaping purposes.

Growing period: Perennial.

Description: The species is usually a shrub 1.5 to 4 m in height but can become a tree up to 20 m or more in height. The root system consists mainly of laterals and fine roots that are dark brown, weak and brittle, and have a corky bark. Plant usually has an erect trunk or multiple trunks, but it may assume a prostrate form and have limbs that layer and become new individuals. The bark is gray or brown, furrowed, fibrous, and moderately thin (about 8 mm). The inner bark is dark cream in color. Stemwood (specific gravity of 1.0) is hard, heavy, and strong. Branches are brittle. The twigs are slender, yellow-green, angled, flattened, or winged. The spirally arranged, elliptic to lanceolate leaves are cartaceous to somewhat fleshy, 2 to 10 cm long, with petioles 3 to 9 mm long. Inflorescences are terminal or axillary panicles of tiny greenish-white flowers grouped in spheroidal heads 3 to 5 mm in diameter. The thin, dry, 5- to 15-mm, two-winged seeds are densely packed into globose clusters (Howard, 1989; Liogier, 1994; Little and Wadsworth, 1964; Nelson, 1996; Pennington and Sarukhan, 1968; Stevens *et al*. 2001).

Geographical distribution: The geographic range of Buttonwood includes the shores of central and southern Florida, including the Florida Keys; Bermuda; most of the West Indies; both coasts of continental tropical America from Mexico south through Central America and northern South America to Ecuador and the Galapagos Islands and Brazil; and tropical West Africa. (Nettel *et al*. 2008; Howard, 1989; Little and Wadsworth, 1964).

Semple (1970) gives the distribution as "along the coasts of west tropical Africa, the Atlantic and Pacific coasts of tropical and subtropical North and South America and throughout the West Indies". Semple notes that pubescent-leaved individuals (as well as the typical glabrous or smooth-leaved ones) are restricted to the northern West Indies, southern Florida, and northern Central America.

Buttonwood has been introduced to Hawaii at least twice and the silver-leaved variety *sericeus* is still commonly planted as an ornamental. The green-leaved variety was introduced to Oahu before 1910, possibly from Florida, and the variety *sericeus* was introduced to Oahu from the Bahamas in 1946. Both forms of Buttonwood have escaped cultivation and established small wild populations on some islands. In contrast to Red Mangrove,

a b c

Figure-1: *Conocarpus erectus*: a) old tree b) flowering stage c) flowers and fruits which was introduced to Hawaii and is now very common and widespread there, Buttonwood has not shown much tendency to spread beyond the initial introduction sites. In Hawaii it is cultivated and sparingly naturalized in coastal areas of Kauai, Oahu, Lanai, and Molokai. (Allen, 1998). The images of *Conocarpus erectus* at various stages of growth are shown in **figure-1**.

Phytochemical studies:

In the HPLC analysis of ethyl acetate and n-butanol extracts of leaves, stem, flowers and fruits of *C. erectus* revealed the presence of gallic acid (**1**), catechin (**2**), apigenin (**3**), quercetin (**4**), quercetin-3-O-glucoside (**5**), kaemferol-3-O-glucoside (**6**), rutin (**7**) and quercetin-3-O-glucoside-6-O-gallic acid (**8**) (Hameed *et al* 2012).

The leaves, stem, flowers and fruits of C. erectus has been studied for the total phenolic, flavonoids, and tannin contents. The total phenolic and tannin contents were estimated by Folin-Ciocalteu's reagent (FCR) and expressed as the number of gallic acid equivalents (GAE) and flavonoid contents were determined by aluminium chloride method and is expressed in rutin equivalents (RE) (Hameed *et al*. 2013).

Methanol extracts of fruits and stems were found to have high phenolic contents equivalent that is 581.1±9.01and 433.9±6.88 mg/g GAE respectively whereas flowers and leaves have moderate phenolic contents (236.78±14.35 and 216.09±14.35 mg/g GAE respectively). Total flavonoids contents in the methanol extract of leaves was found to be 27.0±1.34 mg/g RE followed by methanol extracts of fruits 19.3±0.66, flowers 11.6±0.33 and stems 6.5±0.83 mg/g RE.

The total casein-absorbed tannin content in methanol extracts of stems and fruits have the highest contents equivalent to 158.62±6.89 and 151.72±13.79 mg/g GAE respectively whereas methanol extracts of flowers and leaves have 119.54±7.96 and 68.97±6.89 mg/g GAE , respectively (Hameed *et al*. 2013).

The wood of *Conocarpus erectus* contains conocarpol and 2′-methoxyconocarpol, simple 1,4-diarylbutane-type lignans and conocarpan (**9**), a lignan of the dehydrodi-*iso*eugenol type (Hayashi and Thomson, 1975). The defatted methanol extract of fruits of *C. erectus* was subjected to chromatographic fractionation on silica gel glass column followed by reversed-phase high-performance liquid chromatography-ultraviolet-electrospray ionisation spectrometry analysis (RP-HPLC-UV-ESI-MS). Ellagic acid (**10**), vescalagin (**11**) / castalagin (**12**) isomer and di-(hexahydroxy diphenoyl) galloyl hexose isomer were tentatively identified as major components with many hydrolysable types of tannins on the basis of a comparison of its mass patterns with relevant items in the literature (Hameed *et al*. 2014).

The new trimethoxy-ellagic glycoside (3,3',4'-tri-O-methylellagic acid 4-O-β-glucupyranuronide) (**13**) was isolated from the leaves of *Conocarpus erectus* L. This new compound showed potent inhibitory effect against reactive oxygen species attack on salicylic acid in a dose-dependent manner adopting xanthine/hypoxanthine oxidase assay (Ayoub, 2010).

Biological studies:

Traditionally, this plant has been used for many diseases but it has been evaluated only for a few pharmacological activities which are described below:

Antioxidant activity:

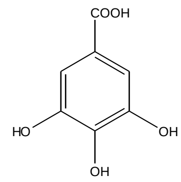
Oxidative stress and free radicals have been involved in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing (Vasi and Austin, 2009). In order to protect the organism against free radicals, consumption of natural source antioxidants is recommended

(Jimenez *et al.* 2009) Medicinal plants that used in ethno-medicine for the treatment and management of many diseases have been investigated for their antioxidative properties (Aruoma, 2003; Semiz and Sen 2007). Secondary metabolites particularly flavonoids from medicinal plants such as *Rosmarinus officinalis, Eugenia jambolana Lam*., *Mytus communis*, *Issopus officinalis, Malva praviflora* etc. exhibited potent antioxidant activity in vitro and in vivo (Usoh *et al*. 2005; Sofidiya et al. 2006; Nwanjo, 2007). Studies reveals that medicinal plants with antioxidative properties might act as free radical scavengers , reducing agents , chelating agents for transition metals , quenchers of singlet oxygen molecules and activators of anti oxidative enzymes to suppress free radical damage in biological systems (Okpuzor *et al*. 2009). The defatted methanol extracts of different parts and its successive fractions of C. erectus were investigated for their antioxidant properties by using phosphomolybdenum and reducing power methods.

Anti-oxidant capacity of methanol extracts of fruits by using phosphomolybdenum method showed higher antioxidant capacity (630.1±5.79 mg ascorbic acid equivalent /g extract) than the methanol extracts of flowers, stems and leaves (579.5±7.58, 570.7±4.37 and 376.3±2.19 mg ascorbic acid equivalent/g extract respectively). In the reducing power activity assay, methanol extract of fruits exhibited the highest reducing power activity (530.3±26.24 mg ascorbic acid equivalent/g extract) followed by methanol extracts of flowers, stems and leaves (479.8±8.75, 479.8±17.50 and 393.9±15.15 mg ascorbic acid equivalent/g extract respectively) (Hameed *et al*. 2013).

Hepatoprotective activity:

Liver is a vital organ play a major role in metabolism and excretion of xenobiotics from the body (Kumar *et al*. 2012). Liver cell injury is



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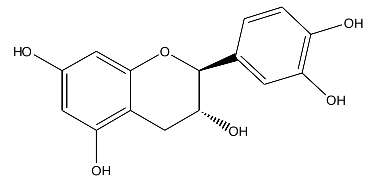

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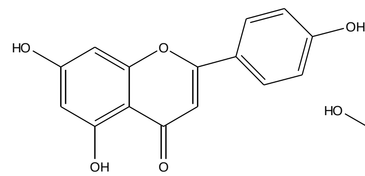

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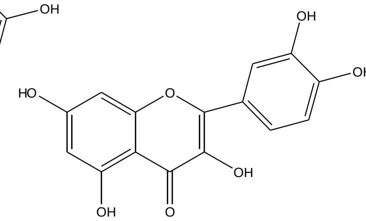

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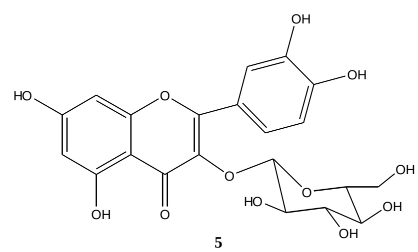

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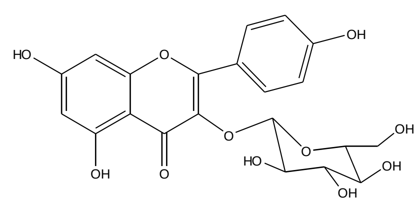

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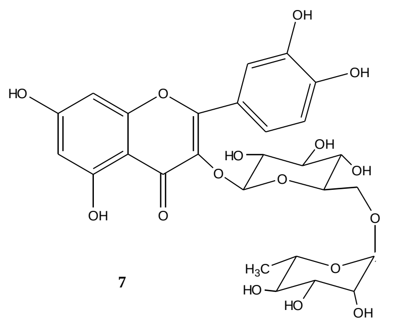
mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, CCl_4, chlorinated hydrocarbon etc.), excess consumption of alcohol, infections, autoimmune/ disorder (Malhotra *et al*. 2001; Kumar et al. 2011). Several medicinal plants have been extensively used in traditional system of medicine for the management of liver disorder (Aniya *et al*. 2002; Gupta, 2006). Plants particularly *Silybum marianum, Andrographis paniculata, Glycyrrhiza glabra, Garcilaria corticata* was reported to possess strong hepatoprotective activity (Bisset 1994). Similarly, *Solanum nigrum* has antiviral activity against Hepatitis C virus (Javed *et al.* 2011).

Compared to normal mice, there is a significant increase in the levels of ALT (P<0.001) and blood urea (P<0.01) in CCl_4 intoxicated mice without affecting the total proteins, albumins, globulins and A/G ratio. Treatment of intoxicated mice with defatted methanol extracts of fruits, flowers, stems and leaves of *Conocarpus erectus* in a dose of 500 mg/kg for two weeks decreased significantly (p<0.5 and P<0.01) the levels of ALT but there is no significant decrease in blood urea level. No change in total proteins, albumins,globulins and A/G ratio were recorded when compared to control group. All the four defatted methanol extracts of *C. erectus* also showed high free radical scavenging activity toward DPPH radical with SC_{50} between 6.47-9.4ug/ml (Hameed *et al*. 2013).



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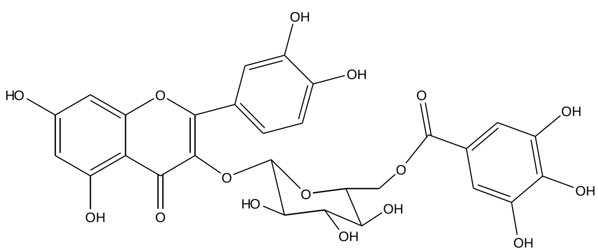

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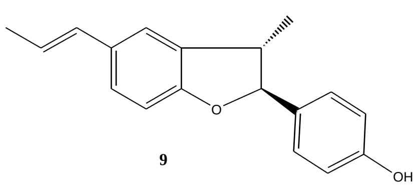

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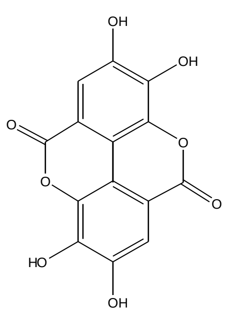

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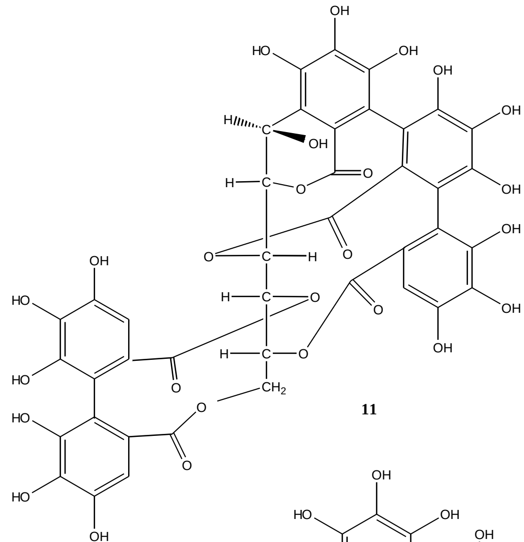

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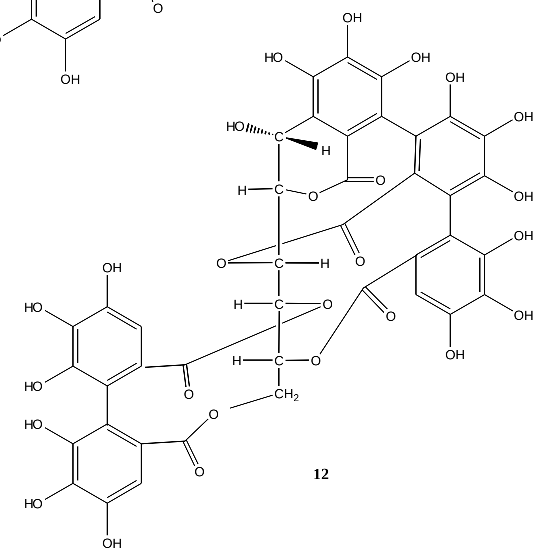

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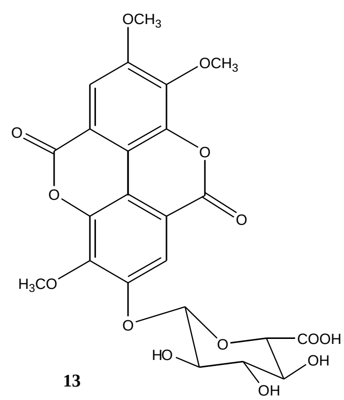

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Anticancer activity:

Cancer is the abnormal growth of cells in our body that can lead to death. Cancer cells usually invade and destroy normal cells (Thakore *et al*. 2011). Plants have proved to be an important natural source of anticancer therapy for several years (Nirmala *et al*. 2013). A number of plants such as *Allium sativum, Bleekeria vitensis, Brassica oleracea, Camellia sinensis, Catharanthus roseus, Ginko biloba, Curcuma longa, Gloriosa superb, Terminalia paniculata, Lavandula angustifolia, Podophyllum peltatum* have been used for the prevention and treatment of cancer (Thakore *et al*. 2011).

The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity. It was estimated that 14 cancer drugs of the top 35 drugs in year 2000 based on worldwide sales were natural products and natural product derivatives (Shoeb, 2006). There is an urgent need to find less toxic and more efficacious new anticancerous compounds.

Cytotoxicity of the ethyl acetate and *n*-butanol fractions of the different parts of *C. erectus* in HepG2 and MCF-7 cell lines by using the sulforhodamine B (SRB). The ethyl acetate fraction of the stems and leaves exhibited high cytotoxic activity toward the HepG2 cell line with IC_{50} = 8.97 and 8.99 µg/ml respectively followed by the ethyl acetate fractions of fruits and flowers with IC_{50} = 10.12 and 11.29 µg/ml respectively. On the other hand *n*-butanol fraction of leaves was found to be active against HepG2 cell line with IC_{50} = 4.89 µg/ml whereas *n*-butanol fractions of flowers, fruits and stems were found to be active with IC_{50} = 9.73, 20.68 and 23.9 µg/ml respectively.

Cytotoxic activity of the ethyl acetate fractions of fruits toward MCF-7 showed IC_{50} = 10.82 µg/ml followed by the ethyl acetate fractions of stems, leaves and flowers with IC_{50} =12.35, 15.84 and 20.86 µg/ml respectively. The *n*-butanol fraction of flowers showed the highest cytotoxic activity with IC_{50} = 7.60 µg/ml followed by leaves (15.04 µg/ml), stems (19.65 µg/ml) and fruits (24.01 µg/ml).

According to the American Cancer Institute (NCI), the criteria of cytotoxic activity for the crude extract is an IC_{50}< 20 µg/ml (Boik, 2001). Most of the fractions showed IC_{50} fall within the NCI criteria, thus these fractions are considered to have a promising anticancer potential.

Antimicrobial activity:

Extracts of *C. erectus* and purified tannins were evaluated qualitatively and quantitatively for their antimicrobial activities. Alcoholic extracts of leaf, stem, fruit and flower were evaluated against Gram-positive, Gram-negative, acid-fast bacteria and fungi by agar disc diffusion method. Tannins were active against the three tested fungi (*Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium. notatum*) with inhibition zones 14.3±0.58, 12.5±1.29 and 13.3± 0.58 mm respectively. Extracts of flower, fruit, leaf and stem were active only against *S. cerevisiae* with inhibition zones respectively 11.3±0.57, 13.3±0.57, 10.3 ±0.58 and 11.0 ±1.0 mm.

Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, were more sensitive than Gram-negative and acid-fast bacteria and their inhibition zones ranged between 21.0 and 23 mm. Inhibition zones of the acid-fast *Mycobacterium pheli* and the tested Gram-negative bacteria (*E. coli*, *Salmonella typhimurium*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) ranged between 11.0 and 18.0mm.

Methanol extracts of different parts of the plant (leaf, stem, fruit and flower), were partitioned by chloroform, ethylacetate and n-butanol. The extracts partitioned with ethyl-acetate were relatively more potent than other solvent extracts of *C. erectus*. MICs and MBCs of these partitioned extracts have been determined against above mentioned organism and it was observed that *S. aureus*, *B. subtilis*, *P. aeruginosa and M. pheli* were relatively the most sensitive and MICs of ethyl acetate partitioned extract were 0.21mg/ml, 0.33 mg/ml, 0.42mg/ml and 0.33mg/ml respectively. This is rather interesting that *P. aeruginosa and M. pheli* are known to be less susceptible to antimicrobials including antibiotics, preservatives, antiseptics and disinfectants (Russell, 1991; McDonnell and Russell, 1999).

The mean MICs of purified tannins against *S. aureus*, *M. phlei* and *E. coli* were 0.054, 0.33 and 0.33 mg/ml, respectively and their respective MBCs were 0.21, 0.67 and 0.67 mg/ml. On the other hand the MICs and MBCs in case of methanol extract were 0.67-4.0 mg and 1.33-8.0 mg/ml respectively (Shohayeb *et al.* 2013). Ellagitannins namely vescalagin and castalagin, purified from an aqueous extract of *C. erectus* were found to be effective in inhibiting pathogenicity of *P. aeruginosa* (Adonizio *et al*. 2008).

Conclusion:

This study revealed that *Conocarpus erectus*, used traditionally for many diseases, has thoroughly been evaluated only for its antioxidant, hepatoprotective, anticancer and antimicrobial activities, while its potential for anti-inflammatory, antispasmodic, antihypertensive, hypoglycemic, urease inhibition and acetylcholine esterase activities is yet unexplored. Similarly, the plant has extensively been investigated for the flavonoids and tannins whereas other classes of secondary metabolites such as saponins, anthraquinones, cardiacglycosides and steroids need to be studied.

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