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Research Article

Isolation and Identification of Phenolic Compounds from *Boswellia ovalifoliolata* Bal. & Henry and Their Free Radical Scavenger Activity

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ABSTRACT

Boswellia ovalifoliolata Bal. and Henry (Burseraceae) is a potential medicinal tree used traditionally in the treatment of ulcers, inflammation, arthritis, obesity and diabetes. The present study aimed to isolate phenolic compounds from stem bark and gum and test for the ability of the extracted phenols of *Boswellia ovalifoliolata*. Total 78 phenolic compounds were obtained when the plant materials were processed through 70% acetone and poly vinyl poly pyrrolidone; and characterized by U.V. Visible spectrometry, High performance liquid chromatography/ electrospray ionization mass spectrometry. Among the isolated phenols, 28 phenolic compounds have been identified based on their retention time and m/z values. These phenols have showed good antioxidant activity the highest hydrogen peroxide (H_2O_2) radical scavenging effect of the isolated phenolic compounds has been recorded at 81.8% when compared to the DPPH; and superoxide ion activities with reference to ascorbic acid. This study illustrate the rich array of phenolic compounds and their free radical scavenging activity of stem bark and gum of *Boswellia ovalifoliolata* could be utility as health beneficial bioactive compounds.

Key words: *Boswellia ovalifoliolata*, stembark, gum, phenolic compounds, Electro Spray Ionization mass spectrometry, hydrozen peroxide

INTRODUCTION

Boswellia ovalifoliolata Bal. and Henry is an endemic, endangered, globally threatened medicinal taxon belongs to the family Burseraceae (Savithramma, 2006). This deciduous medium sized tree occurs at an altitudinal range of 250-600 m on Seshachalam hill range of Eastern Ghats of India. Seshachalam hills are habours large number of endemic, endangered, rare, threatened and key stone species due to its vivid geographical conditions and climatic factors which are favourable for the distribution of unique endemic plant wealth (Savithramma *et al*., 2010). The fresh leaf juice used to prevent throat ulcers (Savithramma and Sulochana, 1998). Decoction of the stem bark 10 – 25 ml per day reduces rheumatic pains (Nagaraju and Rao, 1990). The gum obtained from the trunk which is highly medicated, this gum is sold in the local market by the native tribals as Konda sambrani in Telugu language. Small lumps of fresh light yellow coloured liquid oozes out from the stem and hardens on exposure. Amyrins are the chief constituents of the gum together with resin acids and volatile acids. Shade dried gum is powdered dissolved in water and mixed with curd and given orally to cure amoebic dysentery (Sudhakar, 1998). Gum powder of *Boswellia ovalifoliolata* and *Boswellia serrata* and fruit powder of *Pedalium murex* mixed in equal parts and made into paste and apply externally on the affected part of the testicle to cure hydrocoel. Gum powder mixed with white precipitate of pounded stem of *Tinospora cardifolia* and honey given orally in small quantities (10 ml) two times a day to cure hydrocoel (Vedavathy *et al*., 1995). Equal mixture of gum and stem bark in one tea spoonful given daily with sour milk on empty stomach for a month to cure stomach ulcers (Nagaraju and Rao, 1990). Tribals (Nakkala, Sugali and Chenchu) and local healers of surrounding villages making deep incisions on the main trunk to extract the gum but unknowingly causes damage to immature plants leading to depletion of this species in its natural habitat. Herbal medicines are crude plant drugs used by tribals and rural folk and has also been studied for biological synthesis of silver nanoparticles and antimicrobial activity (Savithramma *et al*., 2011; Savithramma *et al*., 2011; Savithramma *et al*., 2011), phytochemical screening (Savithramma *et al*., 2010), quantification of phytochemicals (Savithramma and Bhumi, 2011), antiulcer activity (Venkateswarlu *et al*., 2012) and antihyperlipidermic activity (Geetha and Ganapathy, 2013).

Phenolic compounds are one of the most diverse groups of phytochemicals that are universally distributed in fruits, vegetable and herbs. Approximately 8000 phenolic compounds have been isolated from natural resources. Polyphenols in nature generally occur as conjugates of sugar, usually o-glycosides, phenolic acids contain two distinctive carbon frame works, the hydroxyl cinnamic and hydroxyl benzoic structures (Santos *et al*., 2003). Although the basic skeleton remains the same, the numbers and positions of the hydroxyl groups on the aromatic ring

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Table 1: Identified phenolic compounds in stembark and gum of *B. ovalifoliolata*

S. No.	Pseudo molecular ion m/z values	Molecular formula	Compound	Stem Bark	Gum
1.	125		Guaiacol G	+	-
2.	169	$C_7H_6O_5$	Gallic acid	+	+
3.	175	$C_6H_5O_6$	Ascorbic acid	+	-
4.	301	$C_{15}H_5O_7$	Quercetin	+	-
5.	315	$C_{16}H_{11}O_7$	Isorhamnetin	+	-
6.	329	$C_{20}H_{25}O_4$	Carnosol	+	-
7.	331	$C_{20}H_{27}O_4$	Carnosic acid	+	-
8.	377	$C_{25}H_{32}O_{13}$	Oleuropein	+	+
9.	388	$C_{21}H_{23}O_7$	Medioresinol	+	-
10.	435	$C_{21}H_{23}O_{10}$	Phloridzin	+	-
11.	488		Coumaroyl hexose	+	-
12.	489		Kuempferol acetylhexoside	+	-
13.	533	$C_9H_8O_4$	Caffeic acid hexoside	+	-
14.	595		Quercetin-3 pentosylhexoside	+	+
15.	121	$C_7H_6O_2$	Benzoic acid	-	+
16.	152	$C_8H_{10}O_3$	Hydroxytyrosol	-	+
17.	153	$C_7H_5O_4$	Protocatechuic acid	-	+
18.	165	$C_9H_{10}O_3$	Phloretic acid	-	+
19.	191	$C_{16}H_{18}O_9$	Chlorogenic acid	-	+
20.	353	$C_{16}H_{17}O_9$	Chlorogenisic acid	-	+
21.	377		5-coumaroylquinic acid	-	+
22.	409		Coumaric acid	-	+
23.	447	$C_{21}H_{19}O_{11}$	Luteolin-7- glucoside	-	+
24.	449		Cyanidin-3-glucoside	-	+
25.	483		1,5-di coumaroylquinic acid	-	+
26.	515	$C_{25}H_{23}O_{12}$	Dicaffeoylquinic acid	-	+
27.	711		Quercetin-7-hexoside-3-hexoside	-	+
28.	1008		Heptamericprocyanidin	-	+

Table 2: Free radical scavenging activity of stembark and gum of *B. ovalifoliolata*

	Sample	8 µl	15 µl	30 µl
Hydrogen Peroxide	SB	46.34%	58.66%	81.8%
	G	18.75 %	27.75 %	71.7%
DPPH	SB	19.22%	19.22%	58.81 %
	G	12.54%	23.12 %	31.13 %
Superoxide	SB	14.52%	35.22%	56.2 %
	G	6.15%	8.25 %	11.15 %

make the difference and establish the variety. Many activities have been reported for most of the phenolic compounds from plants they act as anti-oxidant, anti-inflammatory, anti-viral and anti carcinogenic agents (Visioli *et al*., 2002).

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting attention because they are natural disease preventing, health promoting and anti-ageing substances (Ozyurt *et al*., 2004). Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under oxidative stress conditions. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity (Braca *et al*., 2002). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue injury (Pourmorad *et al*., 2006). Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or a nutritional supplements. Number of plant species have been investigated in the search for novel antioxidants (Koleva *et al*., 2002). Still there is a demand to find more information concerning the antioxidant potential of plant species. Hence the present

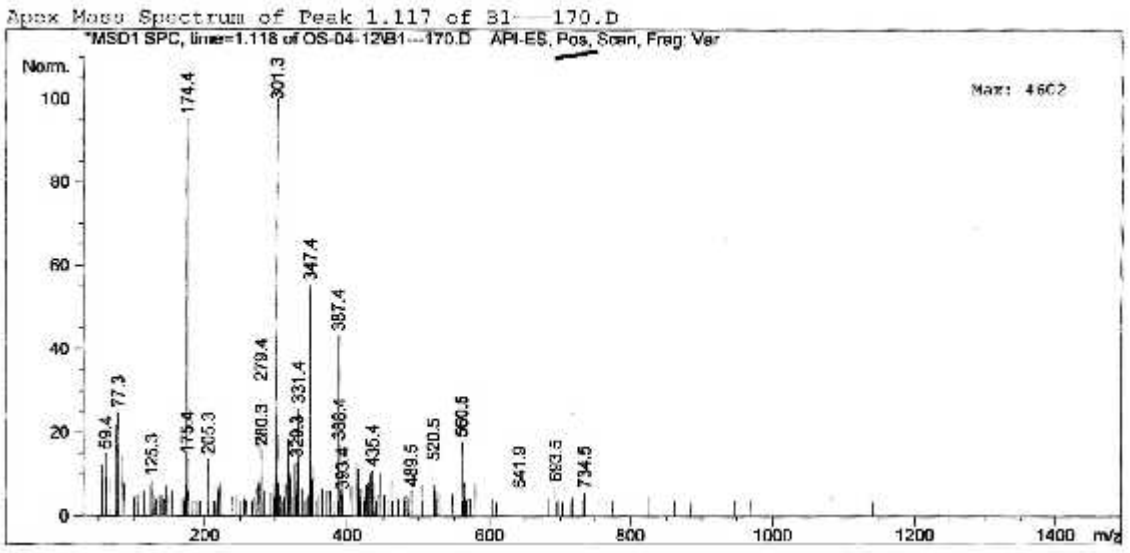


Fig 1: Mass spectra of phenolic extract of stembark of *B. ovalifoliolata* spectra obtained in + Ve ion mode fragmentation

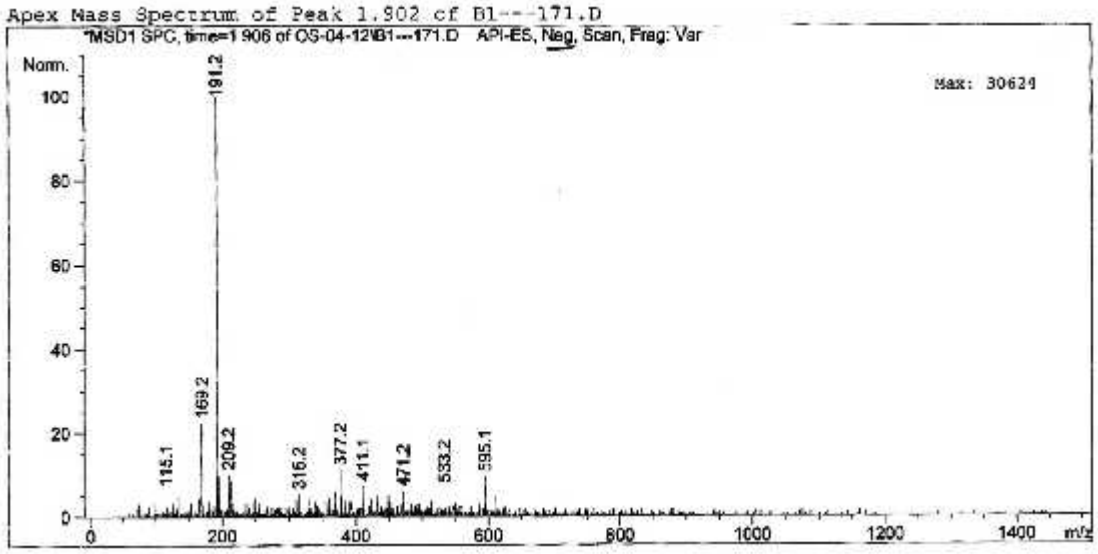


Fig 2: Mass spectra of phenolic extract of stembark of *B. ovalifoliolata* spectra obtained in the – Ve ion mode fragmentation

study to isolate the polyphenols compound and tested the antioxidant activity of *Boswellia ovalifoliolata*.

MATERIAL AND METHODS

Extraction of polyphenols from stembark and gum: Stembark and gum of *Boswellia ovalifoliolata* were collected from Tirumala hills, Chittoor District of Andhra Pradesh, India during July, 2012. The materials were washed thoroughly and shade dried.

30 g of stem bark and gum powders reduced in a mortar and consequently extracted with 500 mL of dichloromethane by ultra-sonication for about 30 min and shaken by vortex for 30 min to remove hydrophilic compounds. Dilapidated powder were extracted with 500 mL of acetone/water (70:30, v/v) by sonication for 15 min and shaking for more 15 min to extract polyphenols. 150 mL of each stembark and gum water extract (pH adjusted to 4.0) was mixed with 5 g of PVPP (30 mg/mL) for 15 min of shaking for adsorption of phenolic compounds to PVPP. The remaining PVPP was re-extracted twice again with 200 mL of fresh extraction solvent for the same period time that was used before. The combined extracts were evaporated at room temperature by rotary evaporation to remove the organic solvent (acetone).

HPLC-ESI-MS/MS analysis: The qualitative study of the phenolic compounds in all samples was performed by HPLC coupled on-line with electrospray ionization (ESI) mass spectrometry. The HPLC system (Agilent 1100 series) consisted of a low-pressure quaternary pump (Agilent 1100 series) and an auto-sampler. A quadropole ion trap mass spectrometer (Agilent 1100) equipped with an ESI source in the positive and negative ion mode and Xcalibur software Version 1.4 (Finningan) were used for data acquision and processing.

Free Radical Scavenging Activity: Hydrogen Peroxide scavenging activity of *Boswellia ovalifoliolata* stembark extract and gum were determined using a modification of the method of Ruch *et al* (1989). Superoxide Scavenging

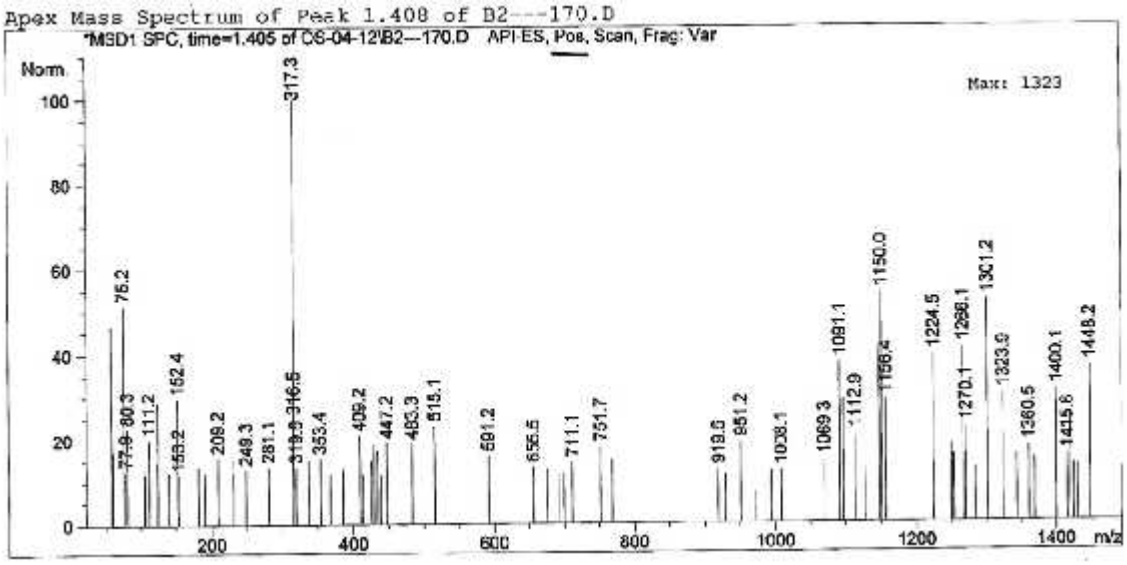


Fig 3: Mass spectra of phenolic extract of gum of B. ovalifoliolata spectra obtained in the + Ve ion mode fragmentation

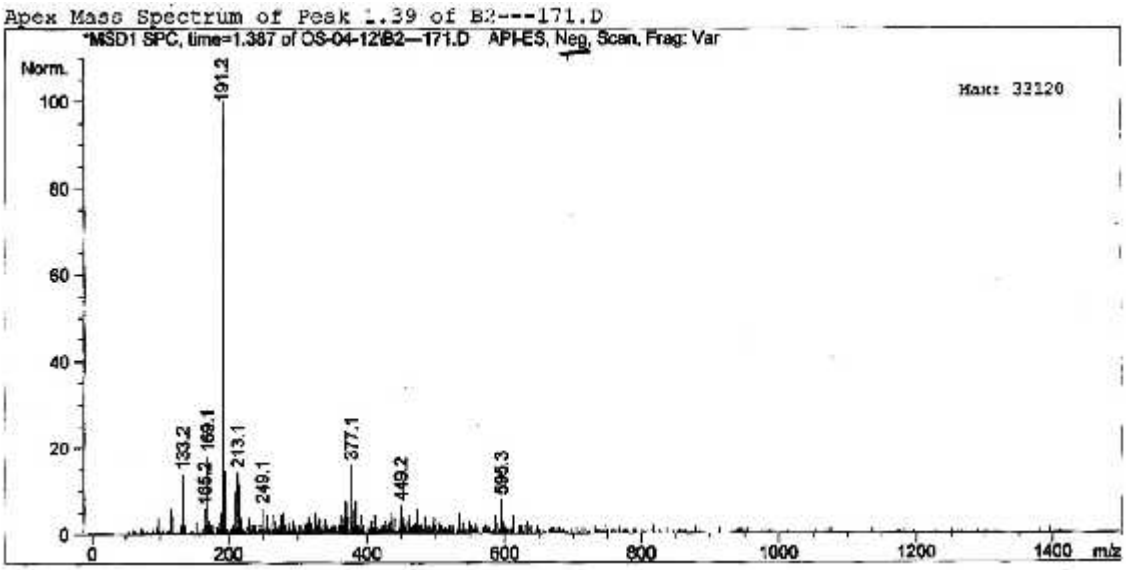
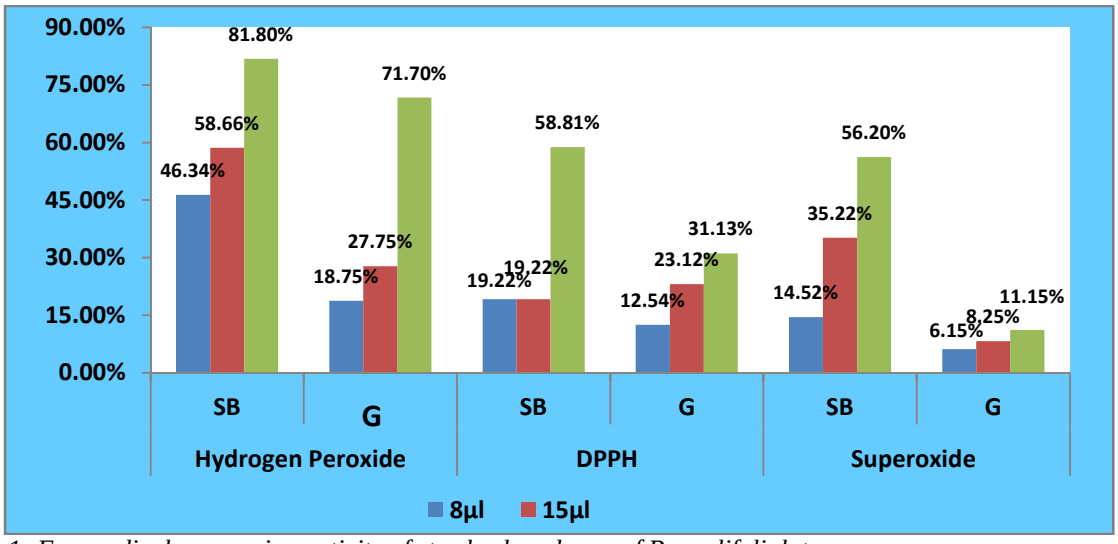


Fig 4: Mass spectra of phenolic extract of gum of B. ovalifoliolata spectra obtained in the – Ve ion mode fragmentation



Graph 1: Free radical scavenging activity of stembark and gum of B. ovalifoliolata

Activity (Grow-Chin and Hui-Yin, 1995), 1,1-diphenyl-2-picrylhydrazyl Radical activity (DPPH) Hatano *et al.* (1998).

RESULTS AND DISCUSSION

Isolation of phenolic compounds: The present study revealed that 78 phenolic compounds were found in the lyophilized samples of stembark (positive mode-22 and negative mode-10) and gum (positive mode-37 and negative mode-9) of *Boswellia ovalifoliolata*. Among these, 28 phenolic compounds were identified by comparing LCMS spectral data with those of literature data. The identification of these compounds was summarized in Table-1 and structures of various isolated phenolic compounds were presented in Fig-5.

Liquid chromatography, electrospray ionization-Tandem Mass Spectrometry (LC-ESI-MS) have shown MS fragmentation data for structural characterization of the extracts of phenolic compounds. Identification has been carried out based on their Pseudomolecular (M-H) ions and m/z values of mass chromatography. ESI operated in negative mode, which is known as a soft and highly efficient ionization method, proved to be an excellent tool for the identification of flavonoid glycosides by providing information on the glycoside molecular masses due to their prominent [M-H] ions and fragmentation products of the aglycone arising from Retro-Diels-Alder reactions. ESI-MS parameters were optimized for the components and the solvent system, respectively to maximum ionization efficiency fragmentation of phenolic acid yield product ions more efficiently at lower collision energies. While flavonols and particularly their aglycones needed higher collision energies to obtain diagnostically relevant product ions (Engels *et al*., 2012).

Negative mode ion at m/z values at 301 is similar to that of quercetin. But this compound seems to be a derivative of quercetin glucoside. The identification of oleuropein was corroborated by detection of the molecular ion at m/z 435 and its phloridzin fragment at m/z 377. These results are similar to *Cotinus coggygria* by Lemonia *et al.* (2009).

The mass spectrum of m/z 435 was formed by the loss of 162 Da and another intense peak at m/z 377 indicative of the elimination of another hexose unit. Among the eluted phenolic compounds, benzoic acid (m/z at 121) vanillic acid (m/z 181) and eudesmic acid (m/z 211) are derivatives of benzoic acid. These are reported from Oak barrels by Regalado *et al.* (2011).

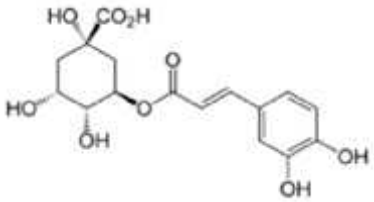
Spectrum acquired in full scan mode displayed intense molecular ion at m/z 301 while is a diagnostic of quercetin derivatives. These fragments showed in higher mass region with a relatively high intensive. But m/z 329 has been reported as carnosol from *Origanum majorana* and Lamiaceae species. According to Hossian *et al*. (2010) m/z 329 is a major fragment of m/z 359 known as methoxy earnosol by subsequent loss of CO_2 molecule.

The ion peak at 1008 was attributed to the doubly charged species of heptameric procyanidin no clear multiply charged species beyond the doubly charged ones were detected, because of the lower concentration of larger tannin molecules (Eddine, 2007). Results of accurate mass measurements are other diagnostic features of these compounds. Nevertheless, with no other information available; it was not possible to identify the structures and natures of other compounds. M/z values represented Gallic acid the loss of carboxylic group of Gallic acid resulted. M/z 125 corresponding to the Gallic acid the more galloyl glucose elutes before Gallic acid, which is constituents with the results reported by Meyers *et al.* (2006). Gallic acid exist in the free form as gallo tannins, these hydrolysable tannins are present in a rich variety of plants and are present in tea, red wine, fruits, beverages and various plant material and is considered to be a generally regarded as safe food additive functioning as an antioxidant in same countries including Japan (Soong and Barlow, 2006). Gallic acid is known to have anti-inflammatory, anti-mutagenic, anti-cancer and anti-oxidant activity (Inouc *et al.*, 1995). M/z values 447 have been identified as luteolin-7-glucoside. By comparing their MS^n dala, retention times and UV spectra with standard compounds, m/z 388 was identified as medioresinol and m/z at 152 as hydroxytyrosol. It is supported by He *et al.* (1997). The compound observed at ESI-MS spectra was characterized by an intense ion at m/z 435. Corresponding to the deprotonated molecule of phloridzin (Fig 1-4).

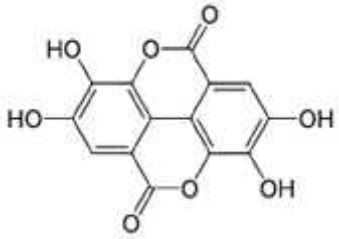
Free radical scavenging activity: Studies on free radical scavenging activity were carried out with isolated phenolic compounds showed that the highest percentage at 81.8 in H_2O_2 in stembark when compare with the gum. The DPPH and superoxide ion activities also found at higher levels in stembark. The percentage of inhibition was increased with increasing concentration of the extracts of stembark and gum of *B. ovalifoliolata* (Table-2 and Graph-1). Similar results were recorded in *Sargassum siliquosum* by Corpuz *et al*. (2013) and *Shorea tumbuggaia* (Ankanna and Savithramma, 2013). They recorded that the scavenging activity of the identified factors in *Sargassum siliquosum*. The maximum scavenging activity (80%) was exhibited by DCM fraction at 10 mg/ml in *S. siliquosum*. Free radicals are formed continuously as normal byproducts of oxygen metabolism during mitochondrial oxidative phosphorylation. Thus the mitochondrion is the main source of free radicals (Fahn and Cohen, 1992). DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extracts of gum and stem bark of *B. ovalifoliolata* showed potential for free radical scavenging activity. Patt and Hudson (1990) described that the antioxidant activities of the individual compounds present in the extracts may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups and other structural features.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, and inside the cell, H_2O_2 probably reacts with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects (Nagavani *et al*., 2010). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to

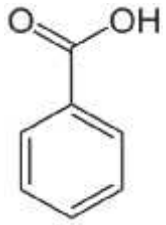
Chlorogenic acid



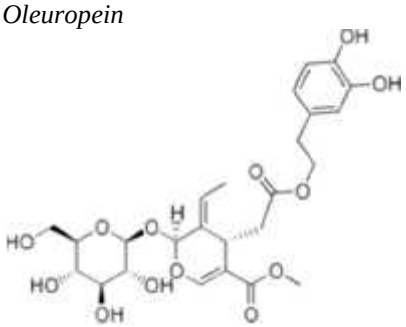
Carnosol



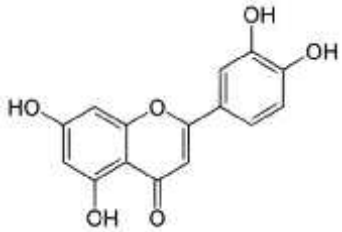
Benzoic acid



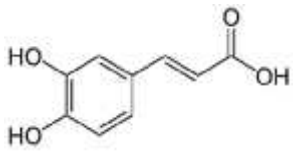
Oleuropein



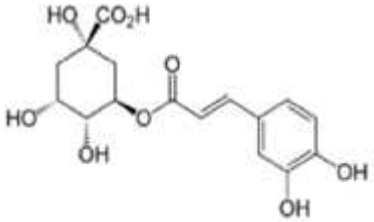
Luteolin



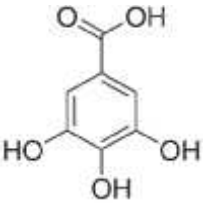
Caffeic acid



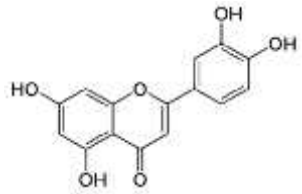
Chlorogenic acid



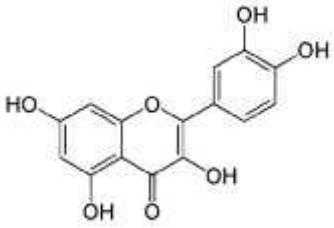
Gallic acid



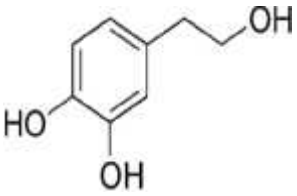
Luteolin



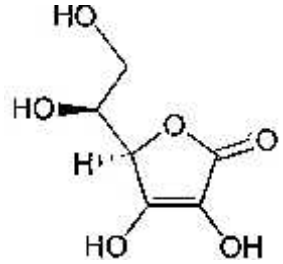
Quercetin



Hydroxytyrosol



Ascorbic acid



Isorhamnetin

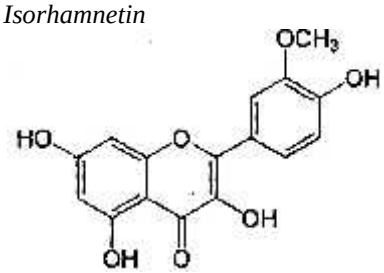


Fig 5: Chemical structures of phenolic compounds detected in B. ovalifoliolata

accumulate. An antioxidant is any substance that retards or prevents deterioration, damage or destruction by oxidation (Bhuiyan *et al*., 2009). These essential chemical substances have the ability to protect humen against detrimental oxidative effects caused by free radicals such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl (OH^-) and DPPH; and collectively known as reactive oxygen species. Kotamballi *et al.* (2002) supported that the extraction with 99.99% ethanol gives not only a considerable yield of extracts but also a high antioxidant activity. Kaczmarski *et al*. (1999) stated that among antioxidative compounds and ascorbic acid shows very strong intensity of antioxidative activities.

Based on the results the stembark is the best source to neutralize the superoxide molecules and alleviate the stress. Hence the extract of stembark may be used as antioxidant agent to get relief from stress conditions.

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