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A comparative study of the *in vitro* antioxidant activities and some phytochemical properties of methanol and ethanol extracts of *Glycyrrhiza glabra* L. roots

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ABSTRACT: *Glycyrrhiza glabra* Linn is a plant used in traditional medicine across the world for its ethnopharmacological value. This present study takes a look at the comparative study of the antioxidant activity and some phytochemicals in the ethanol and methanol extracts *G. glabra* L roots. In antioxidant activity: DPPH scavenging activity, Hydrogen peroxide radicals and thiobarbituric acid reactive species were determined and expressed in terms of standard used for the respective assay. The phytochemical contents of methanol and ethanol extracts of *G. glabra* are 116.27, 84.43mg of phenols, 2265.59, 1132 mg of flavonoids, 965.67 and 1740.58mg of Proanthocyanidin respectively. The % maximum scavenging activity of methanol and ethanol extracts are 28.83%, 29.68% for (DPPH), 77.51%, 78.92% for (H_2O_2) and 11.41%, 26.53% for (TBARS). The present data indicate that ethanolic extract has a higher scavenging capacity than methanol while the methanolic has higher phytochemical contents except for proanthocyanidin. *In vitro* method suggests that *G. glabra* is more powerful against hydrogen peroxide radicals.

Keywords: *Glycyrrhiza glabra*, antioxidant, phytochemicals.

Introduction

Medicinal plants are widely used as alternative therapeutic tools for the prevention or treatment of many diseases. The medicinal value of these plants lies in some chemical substances known as secondary metabolites that produce a definite physiological action on the human body.

Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [1]. Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementia. Antioxidant components such as phenols, flavonoids and tannins are the secondary

metabolites in the plants that exhibit anti-allergenic, anti-microbial, anti-artherogenic, anti-thrombotic, anti-inflammatory and cardio protective effects [2, 3].

Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants [4]. Drugs obtain from plants containing free radical scavengers are known for their therapeutic activity [5]. The screening of Glycyrrhiza glabra extract for antioxidant and antimicrobial activity has revealed a reliable source of new agents to serve the processing of natural product. The present study is the comparative evaluation of ethanol and methanol extracts of G. glabra.

Materials and Methods

Collection and processing of plant material: *Glycyrrhiza glabra* roots were used as plant material. The plant was sourced from Farm Settlement located in Ekpoma, Edo State. It was identified and authenticated at the Herbarium section, Department of Plant Biotechnology, University of Benin with a voucher number of UBH_G 394. Dried roots of *Glycyrrhiza glabra* (Family: Fabaceae) were taken, oven dried and ground in an electric grinder to have coarse powder at pharmacognosy department of Uniben. The powder was stored in an air-tight container at 25℃.

Preparation of methanol and ethanol extracts of *Glycyrrhiza glabra*

16g powdered material of *Glycyrrhiza glabra* roots was put into a plastic container and 800ml of absolute methanol was added, soaked for three days and kept in the laboratory. The content was stirred for 10 minutes after each day for three days. Finally the soaked material of plant was filtered through several layers of muslin cloth one by one for coarse filtration. The filtrate so obtained was evaporated under a temperature of 45 ºC in a water bath. The process of evaporation was continued till little a well concentrated solution was obtained. The paste obtained was subjected to freeze drying to obtain the powdered form. The same procedure was used to obtain ethanol extract.

Determination of DPPH scavenging activity

The free radical scavenging capacity of the ethanol and methanol extracts of *Glycyrrhiza glabra* roots against 2,2- diphenyl-1- picrylhydrazyl (DPPH) radical was determined by a slightly modified procedure of [6]. 0.5ml of 0.3mM DPPH solution in methanol was added to 2ml of various concentrations of the extracts. The reaction tubes were shaken and incubated for 15mins at room temperature in a dark room. Absorbance was read at 517nm and this process was carried out in triplicate. Ascorbic acid was used as the standard control with similar concentration as the test-tubes prepared. A blank solution containing 0.5ml of 0.3mM DPPH and 2ml methanol was prepared and treated as the test-tubes. The % DPPH activity scavenging ac is calculated using

$$\% \text{ inhibition of DPPH radical} = ([A_0 - A_i]/ A_0) \times 100$$

Where A_0 is the absorbance of DPPH radical + methanol

A_i is the absorbance of DPPH radical +sample extract

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective exact concentration of the extract required to scavenge 50% of the DPPH free radical under experimental condition [7].

Determination of hydrogen peroxide scavenging activity

According to [8], a solution of hydrogen peroxide (2mmol/l) was prepared in 50mM phosphate buffer (pH 7.4). Extracts (0.2–1.0 mg/ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

The percentage of H_2O_2 scavenging activity is calculated using the following formula:

% scavenged H_2O_2 = ([A_i - A_t] /A_i]) x 100

Where A_i = absorbance of control
A_t = absorbance of test

Estimation of thiobarbituric acid reactive substances (TBARs)

According to [9], egg yolk homogenate (0.5ml of 10% v/v) and 0.1 ml of each each extract were added to test tubes to make up to 1ml with distilled water. 0.05ml of ferric sulphate (0.07mM) was added to induce lipid peroxidation and incubated for 30mins. 1.5ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 0.05ml of 20% TCA were added and the resulting mixtures were vortex and then heated at 95^0C for 60mins. The generated colour was measured at 532nm and this was done in triplicate. Inhibition of lipid peroxidation % = (C- E) / C x 100

Where C is absorbance value of the fully oxidized control
E is absorbance of the extract.

Determination of total phenolic content

According to [10],. concentrations ranging from 0.2-1 mg/ml of licorice extracts were prepared in methanol. Then, 4.5ml of distilled water was added to 0.5ml of the extract and mixed with 0.5ml of a ten-fold diluted Folin- Ciocalteau reagent. 5ml of 7% sodium carbonate was added to the tubes and another 2ml of distilled water was added. The mixture was allowed to stand for 90mins at room temperature and was read at 760nm. All determination was performed in triplicate with gallic acid utilized as positive control.

Determination of total flavonoid content

According to [11], 2ml of 2% $AlCl_3$ in ethanol was mixed with 2ml of varying concentration of the extracts (0.2-1 mg/ml) in methanol. The absorbance was read at 420nm after one hour incubation at room temperature. Similar concentration of quercetin, the positive control was used. The flavonoid content was calculated as mg quercetin equivalent/g of extract.

Determination of proanthocyanidin content

According to [12], 1ml of 4% methanol solution and 0.75ml of concentrated hydrochloric acid were added to 0.5ml of 0.2-1.0mg/ml of each extract. The mixture was allowed to stand undisturbed for 15mins and the absorbance was read at 500nm. Ascorbic acid was used as the standard.

Statistical Analysis

All the experiment were carried out in triplicates (n =3) and the results were expressed as mean ± Standard Error of Mean (SEM) using Microsoft Excel 2010. The level of significant was analyzed using Graphpad Prism 7.00 with a $p<0.05$ as statistically significant.

Results and Discussion

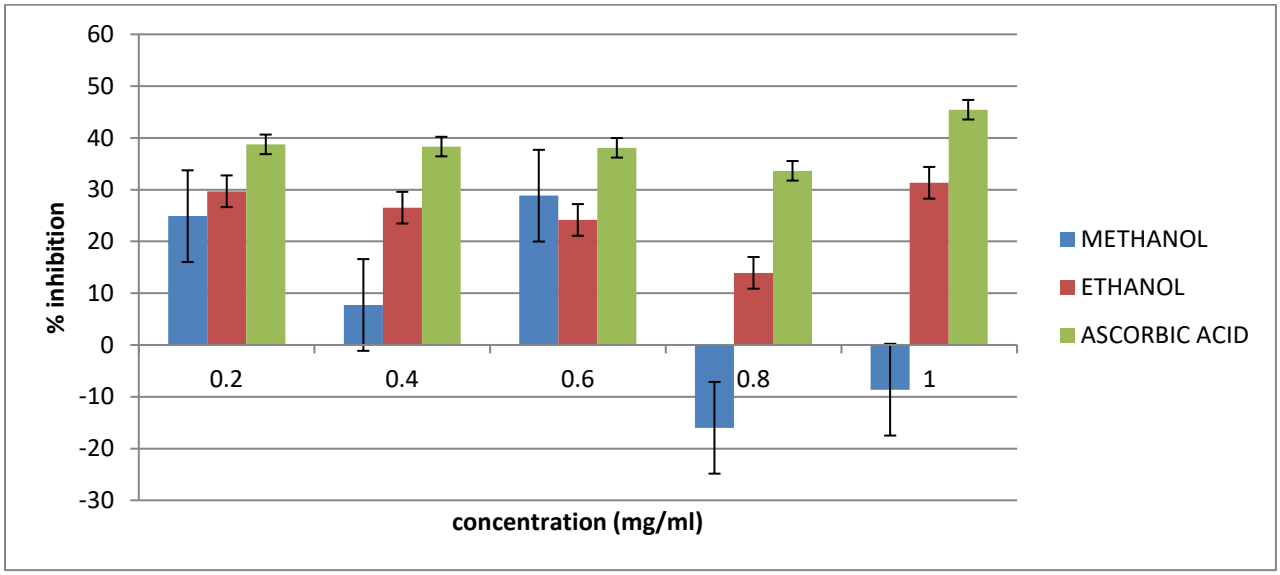

Figure 1: **DPPH scavenging activity of methanol and ethanol extracts of *Glycyrrhiza glabra***

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radical is usually used as a substrate to evaluate antioxidant activity of antioxidants (plant extracts). It involves reactions of specific antioxidant with stable free radical 2, 2-diphenyl-1-picryl-hyrazyl (DPPH). As a result, there is a reduction of DPPH concentration by the antioxidant, which decreases the optical absorbance of DPPH, and it is detected at 517 nm [13]. In DPPH radical scavenging activity, the methanol extract indicated maximum scavenging activity of 28.8% at 0.6 mg concentration of root bark extract. The ethanol extract indicated maximum activity of 31.3% at 1.0 mg concentration of extract and ascorbic acid showed maximum activity of 45.4% at 1.0 mg concentration. The IC_{50} indicates the concentration at which 50% of the test substance is significantly affected. It was observed that methanol and ethanol extracts were unable to scavenge free radicals at 50% inhibition which is in contrast to the work done by [14]. Inhibitory concentration (IC50) value of DPPH scavenging activity of the ethanolic extract of *G. glabra* was found to be 120 (µg/ml) While the IC50 value for ascorbic acid was 84 (µg/ml) respectively. For the negative % inhibition of methanol at 0.8-1.0mg/ml, it could mean that the extract acts as cell division stimulant, promoting cell growth and thus inhibiting cell viability.

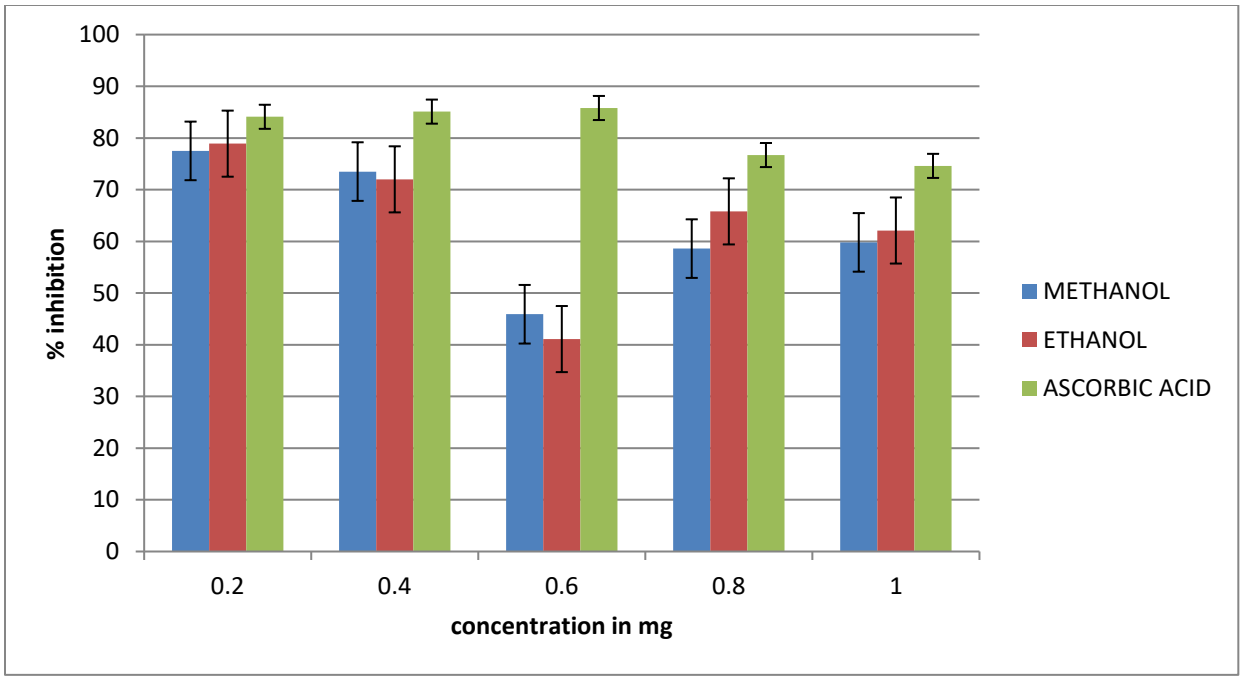

Figure 2: Hydrogen peroxide scavenging activity of methanol and ethanol extracts of *Glycyrrhiza glabra*

Hydrogen peroxide is regarded as a weak oxidizing agent and it is not very reactive, but can cross biological membranes and can possibly be involved in the generation of hydroxyl radicals. H_2O_2 becomes a very important radical that has a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus, removing hydrogen peroxide is very important for the protection of living systems [15]. The hydrogen peroxide scavenging radical effects of *G. glabra* is shown in Table 2 and Fig. 2. The methanol root extract exhibited maximum scavenging activity of 77.51%, ethanol of 78.92% and ascorbic acid 85.82%. The IC50 value of hydrogen peroxide scavenging activity of the control ascorbic acid, methanol and ethanol extracts had IC_{50} values of 0.15mg/ml, 0.42mg/ml and 0.4mg/ml respectively in contrast to IC50 values of 148 (µg/ml) for ethanol extract of *G. glabra* and the IC50 value for ascorbic acid, 96 (µg/ml) respectively[14]. The maximum scavenging activity was found to be 50% of methanolic extract of *Torilis leptophylla (L)* and IC50 value was found to be 130 µg/ml [16]. A higher IC_{50} value indicates lesser free radical scavenging activity. A higher percentage (%) inhibition value indicates higher potent activity. Comparison of the methanol and ethanol extracts with the control shows significant difference.

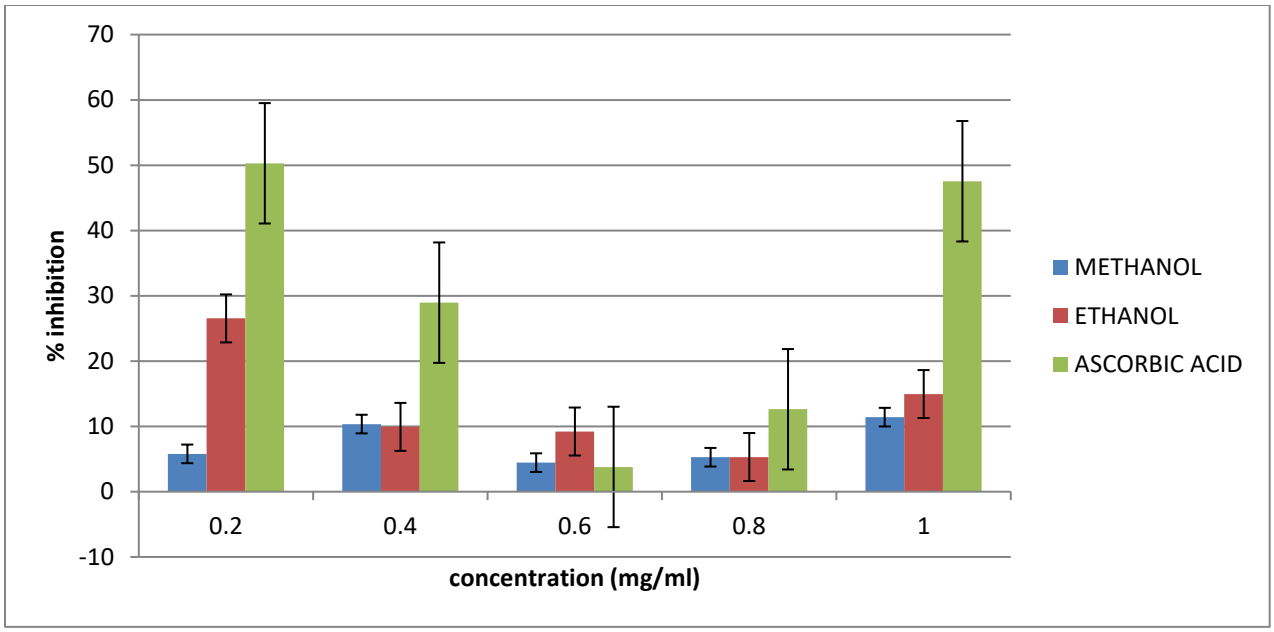

Figure 3: Thiobarbituric acid reactive species of methanol and ethanol extracts of *Glycyrrhiza glabra*

There was a drastic reduction in the % inhibition from 0.2-0.6mg/ml of the extracts and standard and from there they gradually increase. The highest % inhibition in methanol, ethanol and ascorbic acid are 11.414±0.125% at 1.0mg/ml, 26.534±0.168% and 50.290±0.016% at 0.2mg/ml respectively. TBARS measure malondialdehyde present in the sample as well as malondialdehyde generated from lipid hydroperoxide by hydrolytic condition of the reaction [17]. ROS have short half-lives, so they are difficult to measure directly. Therefore, several product of the damage produced by oxidative stress can be measured such as TBARS [18]. However, none of the extracts exhibited scavenging activity greater than the standard i.e ascorbic acid. The radical scavenging values depend on the locality, polarity of the solvents used for extraction and the parts of the plant used in extraction [19].

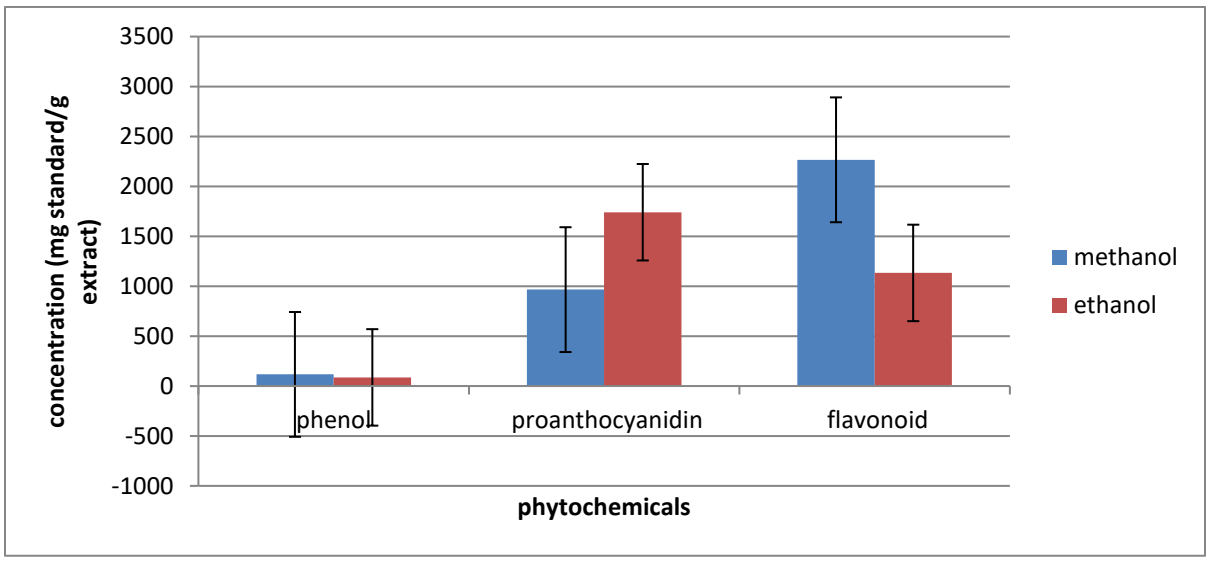

Figure 4: Phytochemicals constituents of methanol and ethanol extracts of *Glycyrrhiza glabra*

Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [14]. The phenol is higher in methanol compare to ethanol, 116.27±0.003 and 84.43±0.000 Gallic acid equivalent/g extract. Flavonoid constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids and DNA, it belongs to the group of polyphenolic compounds, which are classified as flavones, flavonols, isoflavones, and chalcones. The flavones possess a variety of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [14]. The total flavonoid is also higher in methanol, 2265.59± 0.002 while in ethanol was 1132±0.002 Quercetin equivalent/ g.

The proanthocyanidin, the methanol was 965.67±0.002 and in ethanol was 1740.58±0.002 Ascorbic acid equivalent/g. Proanthocyanidin has a protective role against lipid peroxidation and peroxynitrile [20]. Proanthocyanidin was higher in ethanol. It has been discovered that methanol is the best extraction solvent used for the extraction of various active phytochemicals such as flavonoid and phenol [21].

Conclusion

The results obtained from the study that was conducted showed that G. *glabra* is a good antioxidant, particularly a very good hydrogen peroxide scavenger because it contains glycyrrhizin which possesses a good antioxidant activity capable of scavenging free radicals wherever present in the blood circulation in humans. The study indicates high levels of phenol, flavonoid and proanthocyanidin in G. *glabra*, the flavonoid in liquorice has been recorded as the strongest antioxidant known. Therefore, this plant can be developed by the pharmaceutical industries to produce drugs that can improve, treat and manage a number of health related conditions and also for the formulation of cosmetic product for protection against oxidative damage. The ethanol extract has a higher scavenging capacity than methanol while the methanol has higher phytochemical contents. *In vitro* method suggests that *G. glabra* is more powerful against hydrogen peroxide radicals.

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