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# COMPARATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOLIC AND METHANOLIC PLANT LEAVES EXTRACTS OF G*inkgo biloba* L.

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**ABSTRACT**

Phytochemicals are the individual chemicals from which the plants are furnished. These compounds have antimicrobial activity and are the key sources of raw material for both pharmaceutical and aromatic industries. The present study aims to analyze the bioactive compounds which are present in medicinal plant *Ginkgo biloba*. *Ginkgo biloba* which is a very important medicinal plant its leaves were used for this purpose. The extraction procedure was carried out using solvents *viz*; ethanol and methanol. This extract was further used for phytochemical screening. These photochemical compounds may further be used in pharmaceutical and herbal industry for the preparation of different medicines.

**Keywords:** *Ginkgo biloba*, Phytochemical, extract, plant

## 1. INTRODUCTION

*Ginkgo biloba* is prehistoric Chinese tree, present more than 250 million years ago. It is only ongoing member of family Ginkgoaceae (Schmid, 1997). It has been reported that plants contain large number of chemical compounds that are very important against different diseases (Nascimento *et al.,* 2000). *Ginkgo biloba* is considered as living fossil and it contains large number of bioactive compounds i.e. terpenoids, phenolics, flavonoids, tannins, steroids that are used in defense against different pathogens as well as have pharmacological actions (Sati and Joshi., 2011). *Ginkgo biloba* is considered as most sold medicinal plant whose annual sale is estimated from US M$ 450 to over 1 billion US $ (Beek, 2002). The leaves and seeds of *Ginkgo biloba* are used in the preparation of herbal medicines and extracts of *Ginkgo biloba* are used to treat different diseases such as peripheral circulatory and cerebrovascular diseases (Chen *et al*., 1998). Plants contain variety of Phytochemical compounds that have numerous subgroups possessing different bioactivities such as antioxidant, antimicrobial, antivirus, anticancer and so on (Gracelin *et al*., 2012). The use of plant extracts and phytochemicals are of great importance in therapeutic treatments (Fazal *et al*., 2011). The present research work aims to investigate the bioactive compounds which are synthesized by plants so that these compounds may be used in the preparation of different drugs and other herbal medicines

## 2. MATERIALS AND METHODS

### 2.1 Collection of plant leaves

Leaves of *Ginkgo biloba* were collected from Botanical garden of university of the Punjab, Lahore.

### 2.2 Preparation of powder of leaves

Leaves of *Ginkgo biloba* were dried at room temperature. Dry leaves were crushed into fine powder with the help of electrical grinder.

### 2.3 Preparation of extract of plant material

Total weight of plant material was done which was 30 g, and then divided into two equal parts with the help of electrical weight balance. Two solvents were used for the preparation of plant extract viz; methanol and ethanol. 15 g of powder of *Ginkgo biloba* was added into 188 ml of each solvent in a conical flask. It was then placed on electric shaker for 24 hours at 120 rpm and then filtered with whatman filter paper 1. To obtain solvent free extract it was transferred on to a rotary evaporator and pure extract was collected.

**2.4 Qualitative analysis of Phytochemicals present in ethanolic and methanolic extract of *Ginkgo biloba* L.:**

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The following tests were performed on extracts to detect various phytochemicals present in them.

**2.5 Detection of alkaloids**

Following two tests were performed for the detection of Alkaloids.

**2.6 Wagner reagent**

Iodine (1.27g) and KI (2g) it dissolved in 5ml of water and made up to 100ml with distilled water. To a few ml of filtrate, few drops of Wagner's reagent are added by side of the test tube. Formation of reddish brown colour indicated the presence of alkaloids (Omega *et al*., 2009).

**2.7 Hager's reagent**

The Saturated aqueous solution of picric acid was prepared. To a few ml of filtrate, 1 or 2 ml of Hager's reagent are added as result of which yellow colour is appeared which confirmed the existence of alkaloids (Hultin, 1965).

**2.8 Estimation of Amino acid and total Proteins:**

Following two tests were performed according to protocol described by (Roensen and Johnson 1961) for Biuret test and (David and chebbi, 2011) for Ninhydrin test.

**2.9 Biuret test**

Few ml of filtrate was taken and 1-2 ml of Biuret reagent was added. As for as amino acid presence is concerned purple colour should be formed for the presence of proteins, which is not formed reflecting that protein content was absent.

**2.10 Ninhydrin test.**

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added to two ml of test filtrate. Reddish brown ring did not appear which confirmed that protein was absent (David and chebbi, 2011).

**2.11 Detection of carbohydrates**

To know the presence of carbohydrates Fehling's test was performed by the method (Raaman, 2006).

**2.12 Fehling's test**

Copper sulphate (14.88 g) is dissolved in distilled water and made up to 50 ml using distilled water. Potassium sodium tartare (19.22 g) and sodium. Hydroxide (5.55 g) is dissolved in water and made up to 20 ml. One ml of filtrate is boiled on water bath with 1 ml of each Fehling solution A and B. Red colour formation shows that carbohydrates are present (Raaman, 2006).

**2.13 Detection of phenolic compounds**

For detection of phenolics compounds two methods as described by Bray and Thorpe, 2009 using Ferric chloride and Roy *et al*., 2010 using Lead acetate test was performed.

**2.14 Ferric chloride**

The extract (50mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5 % ferric chloride is added. Dark green colour revealed the presence of phenolics compounds (Bray and Thorpe, 2009).

### 2.15 Lead acetate

The extract (5mg) is dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. Formation of bulky white ppt. showed that phenols are present (Roy *et al*., 2010).

### 2.16 Test for flavonoids

Alkaline reagent test was performed by using method described by (Salhan *et al*., 2011) Extracts were treated with 3-4 drops of sodium hydroxide solution. When sodium hydroxide was added then yellow to violet colour was appeared which indicated the presence of flavonines a class of flavonoids.

### 2.17 Detection of tannins

To know the presence of tannins protocol was followed given by (Rumaisa *et al*., 2013). For the detection of tannins 0.5g of dried powder samples were boiled in 20 ml of water in a test and then filtered. To this few drops of 0.1 % ferric chloride was added. When ferric chloride is added brown colour appeared which was a sign of presence of tannin (Aynehchi *et al*.,1981).

### 2.18 Detection of steroids

Salkwoski test was performed for the indication of steroids. 2 ml of methanol and ethanol extract in separate test tube was taken, 2 ml of chloroform and 2 ml of conc. $H_2SO_4$ was added. The solution was well shaken. Appearance of brown layer at interface confirmed the existence of steroids (Edeoga *et al*., 2005).

### 2.19 Detection of Diterpenes

For diterpenes detection copper acetate test was performed according to protocol of (Roopashree *et al*., 2008).

## 3. RESULTS

The results obtained from the screening of *Ginkgo biloba* L. extract in different solvents is presented in Table 1.

**Table 1.** Phytochemical Screening of *Ginkgo biloba* L. leaves extract in different solvents i.e. ethanol and methanol

| S. No | Secondary Metabolites | Ethanol | Methanol |
|---|---|---|---|
| 1 | Alkaloids | + | + |
| 2 | Proteins | _ | _ |
| 3 | Amino Acids | _ | _ |
| 4 | Carbohydrates | + | + |
| 5 | Phenols | _ | + |
| 6 | Flavonoids | + | + |
| 7 | Tannins | + | + |
| 8 | Steroids | + | + |
| 9 | Diterpenes | + | + |

**+** = Indicate the presence **-** = Indicate the Absence

## 4. DISCUSSION

Preliminary phytochemical analysis of plant leaf extracts of *Ginkgo biloba* in ethanol and methanol solvents were conceded to investigate the presence of secondary metabolites such as alkaloids, carbohydrates, phenols, tannins, flavonoids, steroids, diterpenes and carbohydrates. Phytochemical study of bioactive plant extracts in solvents of ethanol and methanol in present investigation has revealed the presence of alkaloids, carbohydrates, phenols, tannins, flavonoids, steroids, diterpenes and carbohydrates.

Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase (Obsorm, 2003). Alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein and biosynthesis of membrane phospholipids (Shelton, 1991). Our findings were correlated with the results of Vimalkumar *et al*., (2014) who also performed the same tests against extracts of leaves of Olea dioica Roxb., for alkaloids detection.

Phenolic compounds are the main constituents of plant extract and have very important function in stabilizing lipid oxidation (Pourmorad *et al*., 2006). It has been found that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and in plant defense mechanisms (Shelton, 1991).

Vimalkumar *et al*., (2014) also reported similar findings using Ferric chloride test for the detection of phenolics compounds in ethanolic leaves extracts of *Olea dioica Roxb*.,

Flavonoids, a class of secondary metabolites, are the most important and most studied phenolic phytochemicals that are widely distributed in plants (Maltas and Yaldiz, 2012). Our results were supported by the findings of Rumaisa *et al*., (2013) who reported same test for the detection of flavonoids in extract of *Leucas aspera* which are in conformity of our findings.

Our findings regarding the screening for steroids and carbohydrates were in conformity with the findings of Roopashree *et al*., (2008) who performed Salkwoski test for the identification of steroids in extracts *Cassia tora, Momordica charantia* and *Calendula officinalis* and Salhan *et al*., (2011) who performed test for the detection of carbohydrates in aqueous and ethanolic leaf extracts of *Clitoria Ternatea*.

The *Ginkgo biloba* L. is an important medicinal plant and have been used worldwide for different purposes and the secondary metabolites which are synthesized in it are of great importance as reported by Roopashree *et al*., 2008 as well. These contents can further be used in the development of different drugs and medicines.

## 5. CONCLUSION

It is concluded from the present investigation that *Ginkgo biloba* L. is an enriched with different secondary metabolites *Viz*; Alkaloids, phenols, steroids, Diterpenes, carbohydrates, flavonoids and many other secondary metabolites. This study may further be used in the formation of different pharmaceutical drugs and herbal medicines.

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