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Research Article

**Phytochemical Analysis
And Invitro Antioxidant
Activity Of
*Dolichos biflorus***

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Abstract

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs. The use of herbs to treat disease is almost universal among non-industrialized societies and is often

more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. The present study focused on the phytochemical analysis and antioxidant activity of the *Dolichos biflorus* leaf in various extracts (chloroform, ethanol and water) were prepared and used for the analysis. Among these extracts, the ethanolic extracts have maximum phytochemicals than compared to other extracts. The extract was further purified using thin layer chromatography.

Keywords: phytochemical analysis, TLC, antioxidant activity, *Dolichos biflorus*

Introduction

Herbal Medicine and herbal plant are used by 75-80% of the world population mainly in the developing countries for primary health care because of better cultural acceptability. The world health organization recently defined traditional medicine (Including herbal drugs) as comprising therapeutic practices that have been in existence for hundreds of years. Herbal drug constitute a major share of all officially recognized system of health in India more than 80% of the world population depend on traditional plant based medicine for primary health care. Plant and plant products are being used as a source of medicinal value. Medicinal plants were assuming greatest importance in the primary health care of individual and community in many developing countries.

Plants and herbs in the Indian system of medicine have been reported to be beneficial against various diseases more than hundreds of plants possess medicinal value. Indian medicinal plants are rich

source of substances that have several therapeutic properties cardioprotective, chemoprotective and other effects [1].

Medicinal plants are assuming greater importance in primary health care of individuals and communities in many developing countries. The medicinal plants are widely used by all section of society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine. The natural products are non-toxic have less side effects and available at affordable cost. Now a days there is a retrieval of interest with herbal based medicine due to the increasing interest in the maintenance of health. Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease; stroke, atherosclerosis and hyperlipidemia are the primary cause of death [2].

Ethno-botany has emerged as an important branch of study, which focuses on the utility of different plant species and their properties as food, medicine and other uses. Plant species of the Himalaya as medicine has been known for a long time. *Macrotyloma uniflorum* (old name *Dolichos biflorus* linn), (fabaceae), is commonly known as horse gram in English [3].

Materials And Methods

Collection And Processing Of Plant

Matured leaves of *Dolichos biflorus* were collected from Tiruchirappalli city during the flowering and fruiting period. The plant leaves were washed with tap water to remove soil and unwanted dust particles. Then the leaves were shade dried, and then powdered by using mechanical blender and stored in air tight bottles.

Extract Preparation: The powdered plant leaves were soaked (10g/100ml) in different solvent (aqueous, chloroform and ethanol), for overnight in rotator shaker.

Preliminary phytochemical screening [4]

Preliminary phytochemical screening of various plant extracts were carried out as per standard procedure.

DPPH assay: The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay which was described by Blois [5].

Hydroxyl radical scavenging assay: The hydroxyl radical scavenging assay was performed by a standard method [6]. The percent inhibition was calculated by comparing the results of the test and blank solution.

Assay of total flavonoid Content: The amount of total flavonoids was determined with aluminium chloride (AlCl₃) according to a known method [6].

Thinlayer chromatography: The plant extract was purified using TLC by the method [7].

Results And Discussion

Horse gram is a legume of the tropics and subtropics, grown mostly under dry-land agriculture. It is grown and used mainly in Southern parts of India. Horse gram is high in protein and iron, which makes it a wholesome food that should be added to our diet on a regular basis. It has calcium, molybdenum, polyphenols, flavonoids, which have high antioxidant capacity and hemagglutinin substance that causes red blood cells to agglutinate. Apart from these, it has carbohydrates, fats, minerals, phosphorus, carotenes and nicotinic acid. Like other legumes, it is deficient in methionine and tryptophan. It is bitter and acrid to taste.

Preliminary Phytochemical Analysis Of *Dolichos biflorus*

Phytochemical analysis of *Dolichos biflorus* was carried out in aqueous, ethanol and chloroform extracts and results are shown in Table 1.

In the aqueous extracts of *Dolichos biflorus* alkaloids, terpenoids, protein and sterols were present. Phenols, tannins, sugar, saponin, flavonoids and quinines were absent. In the ethanol extracts of *Dolichos biflorus* alkaloids, terpenoids, phenols, tannins, flavonoids, quinines, proteins and sterols were present. Sugar and saponin were absent. In the chloroform extracts of *Dolichos biflorus* contains alkaloids, terpenoids, saponin, proteins and sterols were present. Phenols, tannins, sugar, flavonoids and quinines were absent.

Table 1. Phytochemical analysis of *Dolichos biflorus*

Extracts and tests	Extracts		
	Aqueous	Ethanol	Chloroform
Alkaloids	+	+	+
Terpenoids	+	+	+
Phenols & Tannins	-	+	-
Sugar	-	-	-
Saponin	-	-	+
Flavonoids	-	+	-
Quinines	-	+	-
Proteins	+	+	+
Sterols	+	+	+

+ symbol indicates presence : - indicates absence with respect to extractive solvents.

ANTIOXIDANT ACTIVITY

The Figure-1 represents total antioxidant activity in DPPH with three different concentrations. It was found that 250 μ l containing samples have more antioxidant activity compared to the other concentration. Thus the leaf and seed samples of

D. biflorus have more antioxidant activity. The results in Figure-2 indicate that the plant extract is a better hydroxyl radical scavenger than control. From the Figure-3 it was found that leaves ethanol extract of *D. biflorus* have maximum amount of total flavonoid content than control sample.

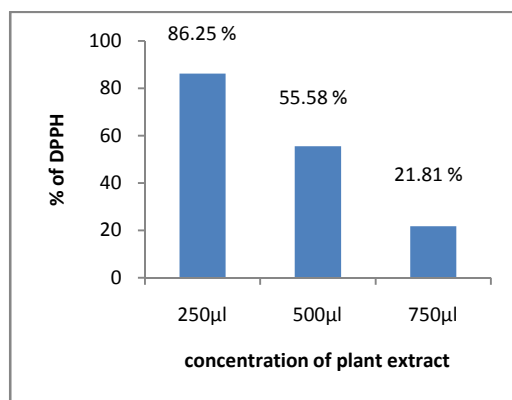


Figure 1. *Dolichos biflorus* % of DPPH in different concentration

H₂O₂ radical scavenger activity

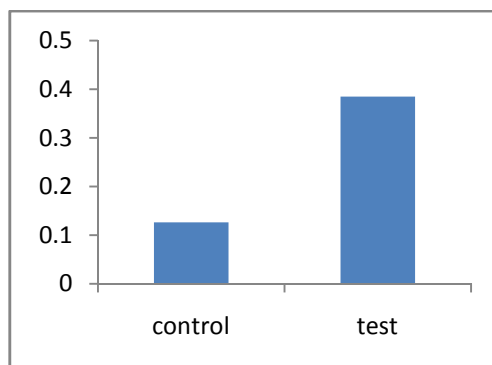


Fig :2 H₂O₂ radical scavenger activity

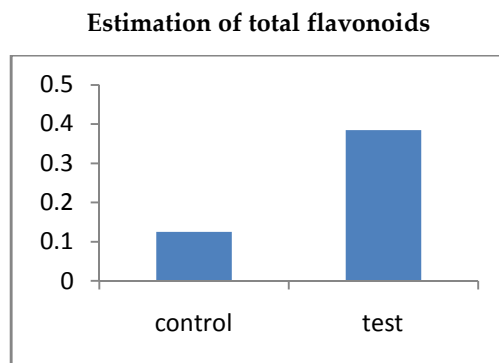


Figure3: Total flavonoid

THIN LAYER CHROMATOGRAPHY

Compound identification was done using silica gel coated thin layer chromatography in water extract. Yellowish green color at visible light mode was present in the tracks of paper identified as compound in the sample.

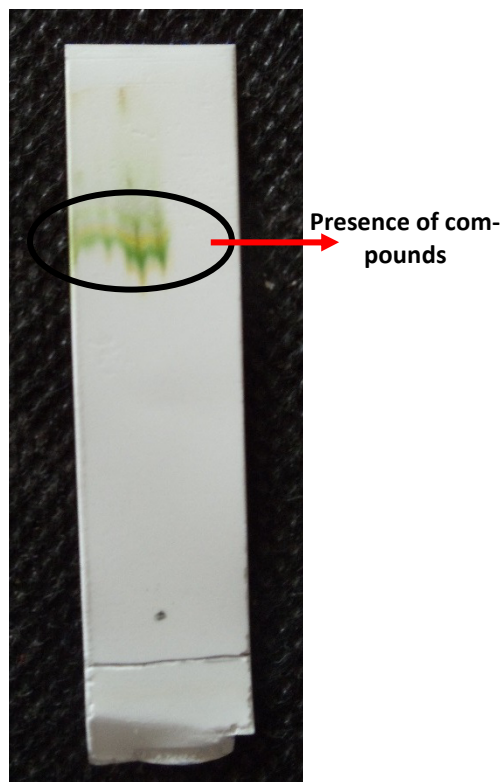


Fig:4 Thinlayer chromatography

Discussion:

The result indicates that combination of plant leaves hold promises as source of pharmaceutically important phytochemicals. Alkaloids present in all three extracts play some metabolic role and control development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins and phenols are known to inhibit pathogenic fungi. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc...^[8]. But free radicals also have significant impact on body's antioxidant defense system and they are capable of generating oxidative stress. So, in the present study, the antioxidant potential of *D.biflorous* was evaluated by examining the different free radical scavenging and total antioxidant activity in DPPH and H₂O₂. Phenolic and flavonoids compounds are very important plant constituents because of their reducing power, scavenging activity and metal chelating property^[9]. The absorption of these compounds rich diet has remarkable effects on human nutrition and health. As depicted earlier, the plant extract has got quite remarkable phenolic and flavonoid content, thus marking it up as a potent antioxidant and free radical scavenger.

Conclulsion

The present study focused on the phytochemical analysis of leaves of the *Dolichos biflorus* in laboratory condition, various extracts (chloroform, ethanol and water) extracts were prepared and the extracts were used for the analysis. Among the three extracts ethanol showed the presence of alkaloids,

terpenoids, phenols, tannins, flavonoids, quinines, proteins and sterols compounds. The presence of phytochemicals in plants is the reason for potent antioxidant and free radical scavenging activity.

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