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IN VITRO STUDY ON ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *CAESALPINIA SAPPAN*

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ABSTRACT

Antioxidants are compounds that protect cell against the damaging effects of the reactive oxygen species (ROS) and reactive nitrogen species(RNS) which can neutralize free radicals and are known to cause damage to lipids, proteins, enzymes and nucleic acid leading to cell (or) tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases. Several medicinal plants have been used by traditional practitioner for treatment of various diseases like inflammatory symptom for a long time without the scientific data supported. *Caesalpinia sappan* is a medicinal plant it possess various immunosuppressive activities, like antibacterial, anti inflammatory, antioxidant and anticancer etc.

INTRODUCTION

Caesalpinia sappan (caesalpiniaceae) is a small thorny tree. It is commonly known as patag.In English known as sappan wood, Brazil wood.The wood was formly used in calico printing of cotton,wool and silk and later on,largely replaced by synthetic dyes.The heart wood is being used to colour wines and meat. The roots of the plant called 'yellow wood' are also used to make yellow dye.The tree was formly cultivated in south-east Asia for the red dye. Brazilin dye is reported to have anti inflammatory activity,obtained from its heartwood(1).*Caesalpinia sappan* is distributed in Tamilnadu, kerala, Karnataka, Andrapradesh and west Bengal(2).The pigment find use in manufacture of facials which are resistant to light heat and water and are non-irritating(3).The mordanted dye with alum displays good fatness towords washing(4).*Caesalpinia sappan* is also a traditional Chinese medicine for activating blood circulation and removing stasis(5).In recent years,the extract of sappan lignum has been found to be a potential immunosuppressive agent(6,7).Many biological activities of *Caesalpinia sappan* have been reported,such as hepato protection(8) immunomodulation(9) hypoglycemic agent activity (10) anticomplementary (11)anticonvulsant (12) anti-inflammatory and antibacterial activity (13,14) xanthin oxidase inhibition(15) aldose reductase inhibition(16)antioxidant activity(17)and protection of the brain(18).Many compounds have already been isolated from the wood of *Caesalpinia sappan* like Flavonoids and phenolics(19) 4-o-methylsappannol,proto sappanin A(20)protosappanin B(21)protosappanin E,brazilin(22).

Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing disease such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful.(23,24).Therefore this study was designed to identify the protective effects of the petroleum ether extracts of *Caesalpinia sappan* against DPPH induced hepato protective activity.

MATERIALS AND METHOD:

CHEMICALS AND REAGENTS USED:

The chemicals used for this experiment was purchased from Merch India Ltd; Mumbai. The chemicals and reagent were of pure analytical grade, HI media.

PLANT MATERIAL

The plant material was collected from Thirunelveli district during the month of March and voucher specimen has been maintained at DKM college and the plant material was authenticated by V. Chelladurai (research officer) Botany (C.C.R.A.S) Government of India.

PREPARATION OF EXTRACTS;

The Arial part of plant materials was collected and dried coarsely powdered and powder was extracted exhaustively with various solvents an increasing polarity viz., methanol, chloroform and aqueous in soxhlet apparatus. Each extract was concentrated to a small volume and allowed to dry. After drying the extracts were stored in an air container for further study.

PHYTOCHEMICAL TESTS:

The qualitative chemical investigations of all the extracts were carried out to check the presence of various phytoconstituents(25).The extracts were kept at-10˚c until they were submitted to the anti oxidant assay.

ASSESSMENT OF INVITRO ANTIOXIDANT ACTIVITY USING 1,1 DIPHENYL 2 PICRYL HYDRAZYL:

The ability of the extract to scavenge DPPH radicals were determined by the method of (27) with minor modifications. A 20µl of aliquot of test extract at different concentrations in methanol was mixed with 0.5 ml of 100 mM methanolic solution of DPPH. After 30 minutes incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm.

The percentage inhibition was calculated using following formula.

Percentage inhibition=(Abs control-Abs sample)×100/Abs control

Comparative in vitro analysis was done using three components namely methanol, chloroform & aqueous solution.

Table 1: Antioxidant activity of *Caesalpinia sappan*

S.no	Name of the Plant	Extacts	Blank	Absorbance at 517 nm	%DPPH Scavenging
1	*Caesalpinia sappan*	**Methanol**	**0.182**	**0.179**	**1.64**
		Chloroform	**0.182**	**0.145**	**20.32**
		Petroleum ether	**0.182**	**0.072**	**60.43**
2	**BHT**	**100 % activity**			

FREE RADICAL SCAVENGING CAPACITY ON DPPH RADICAL:

Measurement of the DPPH radical scavenging activity-The scavenging reaction between (DPPH˚) and an antioxidant(H-A) can be written as

(DPPH˚) + (H-A) → DPPH – H + (A˚)

Purple yellow

Antioxidant react with DPPH˚, which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH˚ radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (26).

Each plant extract was evaluated at 250mg/1 by mixing 0.75ml of each extract with 1.5ml of a freshly prepared DPPH solution (20mg/I); Then, each particular mixture was shaken and left to stand for 30 minutes at room temperature in darkness. After that, each mixture was tested for the DPPH radical scavenging activity by reading the absorbance aat 517nm on an UV-visible spectrophotometer. A blank solution prepared by mixing 0.75ml of ultra pure water with 1.5ml of the DPPH solution (20mg/I) was used and read at the same wave length. In addition, to eliminate the absorbance of the crude extracts at this wave length, blank samples were prepared with 0.75ml of each extract and 1.5ml of

ultra pure water. The DPPH radical scavenging activity percentage was calculated by using the following formula

DPPH radical scavenging activity (%) = [A control – A extract / A control] ×100

Fig: 1 Phytochemical investigation of active constituents in potent extracts of the plant parts

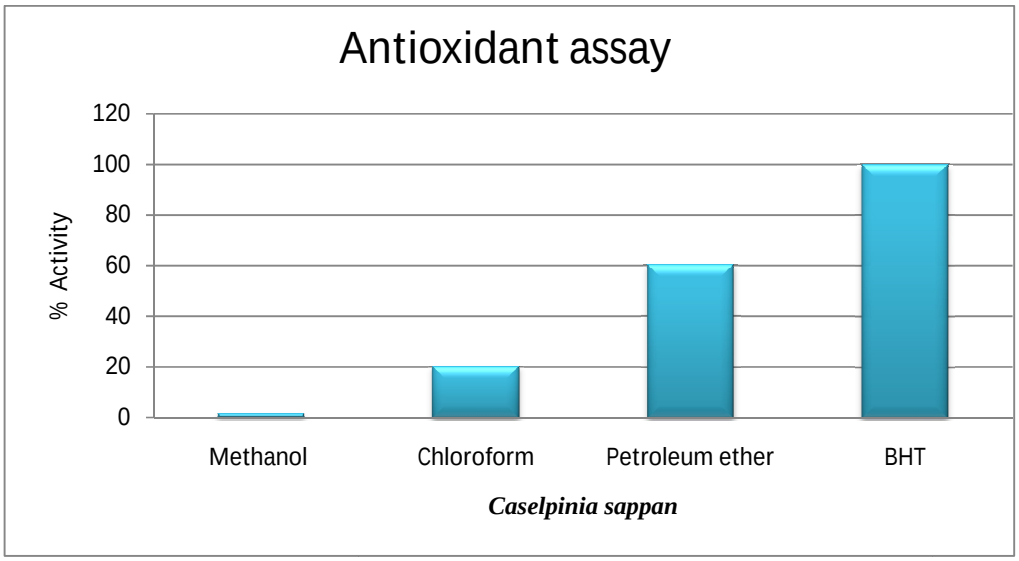

Table 2: PHYTOCHEMICAL TESTS

S.no	Plants	Extracts	Phytochemical Constituents						
			Tannin	**Saponin**	**Flavonoid**	**Alkaloid**	**Protein**	**Steroid**	**Anthra Quinone**
1	***Caesalpinia sappan***	**Methanol**	+	+	+	-	+	+	+
		Chloroform	+	-	+	-	+	+	+
		Petroleum Ether	+	+	+	-	+	+	**+(-)**

(+) present: (-)absent

RESULTS:

Since DPPH assay has been largely used as a quick, reliable, and reproducible parameter to search the in vitro general antioxidant activity of pure compounds as well as plant extracts (28). The antioxidant activity of *Caesalpinia sappan* was evaluated using these polar solvents like petroleum ether, chloroform and methanol .

Phytochemical tests on the methanol and petroleum ether extracts of *Caesalpinia sappan* plants evaluated in this work revealed the presence of tri terpenes, steroids, lactones, tannins and phenols and to these phytocompounds can be attributed the antioxidant activity of these plant extracts.

DISCUSSION:

The result on this work with the *Caesalpinia sappan* family correlates with several results found on the scientific literature .the *Caesalpinia sappan* has been used as a good in vitro and in vivo antioxidant agent because of its powerful scavenger properties of oxygen radicals (29). In general, it is well known that poly phenolic compounds are widely distributed in plant kingdom and they have shown to possess strong antioxidant properties (30,31,32,33)

In conclusion, this study provides evidence that the species caesalpiniaceae have antioxidant properties, as tested through the DPPH method. Therefore, these species may have great relevance in the prevention and therapies of diseases in which oxidants or free radicals are implicated. In additional, these plants can be good candidates for further phyto chemical and chromatographic studies to isolate and fully characterize the compounds related to this *in vitro* biological activity.

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