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Research Paper

Evaluation of Cleome Gynandra For Its Chemical Composition, Antioxidant Potential And Detection of Flavonoids Using Thin Layer Chromatography

Microbiology

KEYWORDS : Cleome gynandra, solvent extracts, phytochemicals screening, antioxidant activity.

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ABSTRACT

Cleome gynandra a well known medicinal plant with known anti-inflammatory activity was studied for its antioxidant property and preliminary detection of compounds responsible for its antioxidant potential. Non polar and polar extracts of Cleome gynandra was subjected to various preliminary phytochemical screening for the detection of phytochemicals. Various in vitro antioxidant method like DPPH radical scavenging assay, Reducing power assay and Metal chelation assay was conducted to evaluate its antioxidant potential and active principle responsible for antioxidant activity was screened using Thin Layer Chromatography. Preliminary phytochemical analysis revealed the presence of flavonoid, alkaloid, terpenoid, steroid and tannins in different solvents. Both methanol and acetone extract proved the best antioxidant activity when compared with other extracts. And Thin Layer Chromatography guided detection showed that active component of the methanol extract was found to be flavonoid and needs further compound identification. Thus it could serve as antioxidant drug in future.

INTRODUCTION

Many reports presents the anti-inflammatory properties of phenolics and flavonoids [Zhang L, Ravipati AS, 2011 and Talhouk R, Karam C, 2007]. TNF-α and nitric oxide (NO) which are pro-inflammatory molecules are believed to be responsible for modulating inflammation besides their crucial role in immune-inflammatory response. Cell death and tissue damage are also due to these pro-inflammatory molecules. NO reacts with the free radicals such as superoxides to produce peroxynitrite, and leads to irreversible damage to cell membranes [Lee MS, Yuet-Wa JC, 2005 and Wang C, Schuller Levis GB 2004]. These cell death, bio molecule damage and tissue damage can be reduced by the use of antioxidants which scavenges oxidants and reduces the formation of free radicals. Since, synthetic antioxidants have many negative effects, in the present study search for effective natural antioxidant was conducted. Cleome gynandra also called Cleome pentaphylla, Gynandropsis gynandra is a common annual vegetable belongs to Capparaceae family. The plant grows up to 60 cm height in tropical and subtropical regions. It is known for its indigenous use in traditional medicine [Borgio JF, Thorat KP, Lonkar AD, 2008]. The leaves of the plant were used for the treatment of various health related problem since ancient time. The leaves are well known for its Anti inflammatory activity [Mule SN, Ghadge RV, 2008 and Narendhirakannan RT, Kandaswamy M, Subramanian S, 2005]. Analagesic, disinfectant and antiseptic properties of the plant are reported. Thread worm infection, conjunctivitis, convulsions are treated by the use of leaves of *Cleome gynandra* [Anbazhagi T, Kadavul K, Suguna G, Petrus AJA, 2009]. The plant has also been reported to have insecticidal, antifeedant, repellent and antimicrobial properties [Akhtar MN, 1990, Malonza MM, Dipeolu OO, 1992, Pipithsangchan, S, 1993,]. Since the plant is known for its anti-inflammatory property the leaves of the plant was evaluated for its antioxidant property.

Materials and methods

Chemicals

Butylated hydroxyl anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), ethylenediaminetetra acetic acid (EDTA), potassium ferricyanide, ferric chloride, ferrozine and ascorbic acid were obtained from (Sisco Research Laboratories, Mumbai, India). Solvents and reagents were obtained from Merck Ltd., Mumbai, India.

Plant material and Extraction

The plant *Cleome gynandra* was collected in and around Mysore. The leaves were separated, washed under running tap water and dried under shade at room temperature. The dried plant material was weighed and powdered. 20 g of powder was extracted serially with 100 ml of non- polar and polar solvents (hexane, chloroform, acetone and methanol) at room temperature for 24 h using rotary shaker. The extracts were filtered using Whatman No. 1 filter paper and concentrated by rotary flash evaporator. The crude extracts were stored at 4^{o} C prior to analysis.

Phytochemical screening

All the extracts of *Cleome gynandra* were analysed for the presence of phytochemicals such as flavonoids, alkaloids, terpenoids, steroids, glycosides, phenols, saponins and tannins according to method described by Harborne JB, 1973.

Evaluation of antioxidant activity

DPPH radical scavenging assay

The method described by Smith RC, Reeves JC and Dage RC, 1987 was followed for measuring DPPH radical scavenging activity of the extracts. Different concentrations of test samples were treated with 300 μM of DPPH solution in 96-well microtitre plates, incubated at room temperature for 30 min in dark and the absorbance was measured spectrophotometrically at 515 nm. Percent radical scavenging activities of test samples were determined by comparison with solvent and positive control. Ascorbic acid was used as positive control. IC50 values denoting the concentration of sample which is required to scavenge 50% DPPH free radicals was also determined. The radical scavenging percentage of the extracts was calculated using the following formula.

DPPH radical scavenging effect (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] X 100.

Where Absorbance of control is the absorbance of DPPH radical + methanol and Absorbance of sample is the absorbance of DPPH radical + test sample/standard. The extract concentration providing 50% inhibition (IC50) was obtained by plotting inhibition percentage versus extract concentration.

Reducing power assay

The method described by Yen GC and Chen HY, 1995 was followed for determining total reducing power ability of the test samples. Different concentration of test samples were mixed with 0.2 M phosphate buffer (pH 6.6) and potassium ferricyanide (1%). The reaction mixture was incubated for 20 min at 500 C. This was followed by the addition of Trichloro aceticacid (10%) and centrifugation at 3000 g for 10 min. The upper layer of the solution was then mixed with ferric chloride (0.1%) and 1.5ml

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of distilled water. The absorbance was measured at 700 nm in a spectrophotometer. BHA was used as a positive control.

Metal ion chelating activity

The method described by Dinis TCP, Madeira VMC and Almedia MLM 1994with minor modification in microtiter plate was followed for determining the chelating effect on ferrous ions. 100 µl of different concentration of test samples were added with 135 µl of distilled water, 2 mM of Ferric chloride and 5 mM of ferrozine. The reaction mixture were mixed and incubated for 10 min at room temperature. The absorbance was measured at 562 nm at room temperature. EDTA was used as a positive control. The ferrous ion-chelating ability was calculated using following formula:

Chelation (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] X 100.

Where Absorbance control is the absorbance of ferrozine + ferric chloride and Absorbance sample is the absorbance of ferrozine + ferric chloride + test sample/ standard. The extract concentration providing 50% inhibition (IC50) was obtained by plotting inhibition percentage versus extract concentration.

Detection of Flavonoids

An aliquot of methanol extract (1 mg/mL, 3 µL) was directly deposited (as spots) onto the TLC sheets. TLC sheets were developed in a presaturated solvent chamber with n butanol : acetic acid : water (4:1:5) as developing reagents until the solvent front reached 1 cm from the top of plates. The developed TLC sheets were then removed from the chamber, air-dried for 30 min, and sprayed with Aluminum trichloride (10 % in methanol) for detection of flavonoids [Avalaskar AN, Itankar PR, Joshi VS, Agrawal M, Vyas J, 2011]. Each TLC sheets was also monitored under UV light at 254 and 366 nm. Since flavonoids possess antioxidant property detection and confirmation of flavonoids was done.

Statistical analysis

Results were expressed as mean±SD. The statistical comparison among the groups were performed with one way ANOVA at $p<0.05$ significant level (SPSS software version – 16).

Results and discussion

In the present study, the phytochemical screening and antioxidant activities of non-polar and polar extract of *Cleome gynandra* were performed. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, steroids, terpenoids, phenols, cardiac glycosides, tannins and saponins. The phytochemical constituents of *Cleome gynandra* investigated are summarized in (Table: 1).

Table 1. Phytochemical screening of Cleome gynandra

Phytochemicals	Test name	HE	CE	AE	ME
Flavonoids	Sodium hydroxide test	-	+	++	++
Alkaloids	Dragendorff's test	-	++	+	+
Steroids	Salkowski's test	+	+	-	+
Terpenoids		+	-	-	-
Glycosides		-	-	-	+
Tannins	Ferric chloride test	-	+	++	+
Saponins	Foam test	-	-	+	-
Phenols		-	+	+	++

- Absent, + present

Where HE : Hexane Extract, CE : Chloroform Extract AE : Acetone Extract, ME: Methanol Extract

Free radical scavenging ability of different extract of *Cleome gynandra* was evaluated using DPPH radical. Ascorbic acid was used as a positive control. It was determined that methanol extract of *Cleome gynandra* possessed higher radical scavenging ability of 73.57 % at50 µg/ml and was compared with standard where it showed 94.88 % activity. Where as hexane extract, chloroform extract and acetone extract showed 47.71 %, 55 % and 70.28 % scavenging activity respectively (Figure 1).

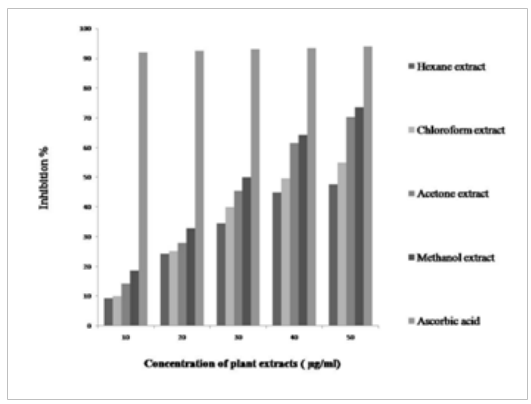

Figure 1 : DPPH radical scavenging assay of Cleome gynandra

Acetone extract showed better reducing power ability and was followed by methanol extract, chloroform extract and hexane extract (Figure 2).

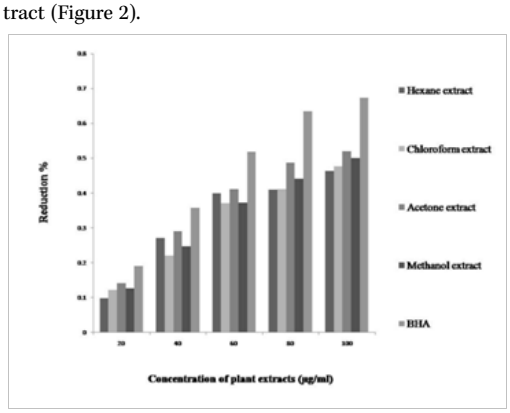

Figure 2 : Reducing power assay of Cleome gynandra

The chelating ability of *Cleome gynandra* was determined in which methanol extract showed the highest chelating ability of 65.75 % at 100 µg/ml. Acetone extract, chloroform extract and hexane extract showed 63.28 %, 36.90 % and 25.49 % chelating ability. Where as EDTA showed 89.3% chelating ability (Figure 3).

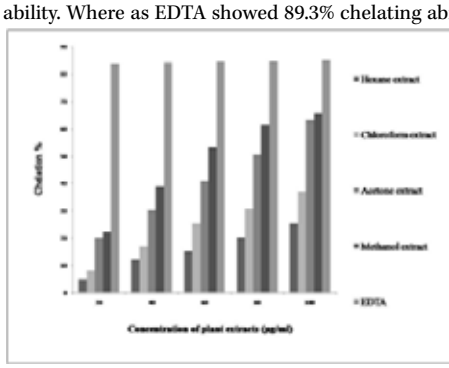

Figure 3: Metal chelation assay of Cleome gynandra

Methanol extract proved to possess better antioxidant principal through various in vitro antioxidant assays. Methanol extract which was subjected for TLC for detection of flavonoids is presented in Figure 4.

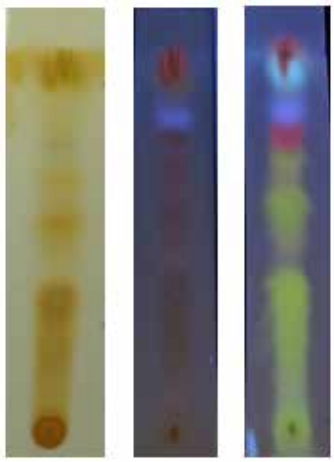

Figure 4: TLC Sheet of *Cleome gynandra* (methanol extract) visualized (A) with iodine, (B) under UV 366 nm, (C) sprayed with AlCl3

DPPH is a stable free radical and it is used to determine the antioxidant ability of plant extracts or compound more rapidly. Methanol extract of Cleome gynandra was observed to possess better DPPH radical scavenging ability. This is due to ability of methanol extract to donate hydroxyl radical to DPPH and make it stable. Ability of a compound or plant extract to reduce Fe3+ to Fe2+ indicates its potential antioxidant activity [Meir S, Kanner J, Akiri B, Hadas SP, 1995]. Reducing power capacity of the extract increased with increase in concentration. The greater reducing power of the acetone extract is may be due to presence of hydrophilic polyphenolic compounds [Karawita R and Siriwardhana N, 2005]. The chelating agent reduces the redox potential and stabilizes the oxidized form of metal ions. Thus the methanol extract acts as a better chelating agent when compared with other extracts of Cleome gynandra. The chromatogram revealed the presence of at least 7 spots. Among these flavonoids were confirmed by yellow fluorescence on spraying with aluminium trichloride solution [Avalaskar AN, Itankar PR, Joshi VS, Agrawal M, Vyas J, 2011].

CONCLUSIONS

The present work confirmed the presence of various secondary metabolites in different solvent extracts and among these different solvent extracts of *Cleome gynandra* evaluated for their antioxidant activity methanol extract showed the strong antioxidant activity and revealed the presence of flavonoid with the aid of Thin Layer Chromatography. These flavonoids act as free radical scavengers. And thus, further research is required to isolate and identify this antioxidant principle which could be a further antioxidant drug.

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