ISSN: 2454-3659 (P),2454-3861(E)
Volume III, Issue 12, December 2017
International Journal of Multidisciplinary Research Centre
Research Article / Survey Paper / Case Study

PHARMACOGNOSTICAL AND PHARMACOLOGICAL PROFILE OF CONYZA BONARIENSIS

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ABSTRACT

The present research work inflicts the importance of traditional medicines in recent days, and it also gives the knowledge of standards & parameters to improve quality of herbal drugs. Some parameters are given in the research are macroscopic and physico- chemical parameters, such as ash value, extractive values with different solvents, and for evaluating efficacy of crude extracts biological activity has been performed such as antifungal activity, antibacterial activity

INTRODUCTION

Focus on General Introduction

On the prospect of history natural products from the plants have played a major role in the life of human beings regarding for food source and for medicinal products (Leland et al., 2006). Early documents about the use of medicinal plants are sparse. History of mankind describes a strong relationship between man, plants and the drugs derived from plants. It is a fact that majority of the world population still depend upon plant for the treatment of different kinds of ailments. The Greeks are considered to have a great knowledge about the uses of herbs. Babylon about 2000 B.C. gives instruction for the preparation and administration of medicinal herbs by the early first century A.D. Greek herbalist listed 500 plants with their medicinal properties. Romans also have a great deal with medicinal herbs. About 200 herbs were introduced to Britain by the invading Romans (Peter et al., 1994). Herbal remedies have got a popular position among the patient with medical problems like

arthritis, diabetes, cancer, depression, eczema, insomnia, and cardiac disorders. The role of plants in health care system is still under estimated. Unani system of Pakistan, Ayurvedic system of India and Chinese herbal system are the pillars of modern medicine system.

Historical Prospects of the Medicinal Plant

Traditional herbalism is a popular system along with modern health care system. There is a need to learn more about the efficacy, adverse effects and the quality of phytomedicines remedies. More Clinical trial evidences about the efficacy and safety of herbal remedies. along with their relevant pharmacological activities are required to be demonstrated (Fencial et al.,1993). Knowledge of the medicinal plants has been mostly inherited. Preservation and spreading this knowledge along with conservation, cultivation and assessment of all medicinal plants has become important for mankind existence.

PLANT INTRODUCTION

Plant Profile of *Conyza Bonariensis*

Conyza (horseweed, butterweed or fleabane) is a genus of about 50 species of flowering plants in the family Asteraceae, native to tropical and warm temperate regions throughout the world, and also north into cool temperate regions in North America and eastern Asia. The genus is closely related to *Erigeron* (also known as fleabanes).The species are annul or perennial herbaceous plants, rarely shrubs, growing to 1-2 m tall **(Fig. 1)**. The stems are erect, branched, with alternate leaves. The flowers are produced in inflorescences, with several inflorescences loosely clustered on each stem.It is met with on the tropical Himalaya from Nepal to Shikhim, altitude to 4000 feet extending to Assam, Khasia Hills, Chittagong Burma and the Straits (Watt et al., 1962).

Description of *Conyza Bonariensis*

Erect perennial, stem rough branching extensively at the base of the plant with tapered leaves covered in stiff hairs, 20-75 cm in height. Erect stems with stiff hairs, branching extensively at the base, decreasing upwards and stems can be 20-75 cm in height. Narrow lanceolate leaves are grey to green in colour, measuring 2-6 cm in length, coarsely toothed and covered in fine hairs. Upper leaves are smaller and linear. Flowers are numerous on poorly arranged pyramidal panicles (much branched inflorescence). The capitulum (flower head) is greater than 2 mm in diameter and looks in fact like a flower bud. Flowers occur at the ends of the branches. Surrounding each flower are involucral (bell-shaped leaf-like) bracts 3-5 mm in

length, the inside of each bract is white sometimes tinged purple or red. The cypsela (fruit) is a linear shaped seed approximately 1.5 mm long, straw colored, covered in hairs with 16-20 noticeably longer at the top that are white or pink **(Fig. 2)**. Flowering is in spring-autumn. (Jessop et al.,1981).

Botanical Classification *of Conyza bonariensis.*

Botanical Classification *of Conyza bonariensis*	
Kingdom	Plant
Sub kingdom	Vascular plants.
Super division	Seed plants.
Division	Flowering plants.
Class	Dicotyledons.
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Conyza Less,-horseweed
Species	*Conyza bonariensis* (L) Cronq. (Asthma weed)

Fig.1 Showing the full grown Plant of *Conyza bonariensis*

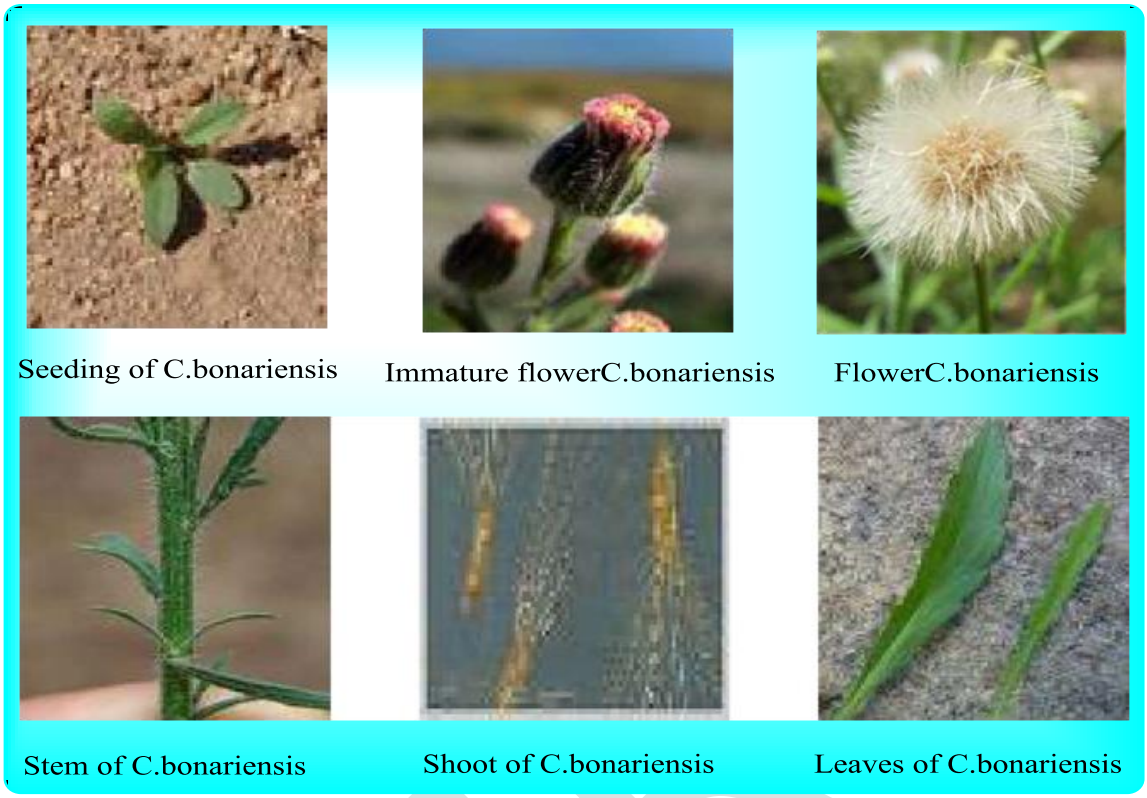

Fig.2 Showing the morphological and physical state of plant *Conyza bonariensis*

EXPERIMENTAL

MATERIAL & METHODS

PLANT MATERIALS

Collection and Identification of Plant Material

Plant *Conyza bonariensis* was collected from Kheri Bawli Delhi India. The plants were identified by Prof. Dr.Anju Pal, Horticulture department, panthnagar university, Panthnagar, Uttarnchal, India.

Solvents and chemicals

All the solvents used for extraction and isolation like methanol, , chloroform, n-hexane, ethyl ecetate, ethanol, proponol, n- butanol Vanillin, silica gel (70-230 mesh) and TLC aluuminium sheets 20 x 20 cm, Silica gel 60 F_{254}. , were imported from Merck KgaA Darmstadt Germany. Sephadex LH-20 25-100|jm FlukaChemie GmbH (9041-37-6).

Macroscopic Identification

Thin section were made with the help of blade, stained and mounted following the usual plant micro-techniques. For the study of isolated cells and tissues, small pieces of leaves, roots, stem, were taken. Washed and mounted in glycerine. The anatomical sketches were made with digital camera.

Quantitative Leaf Microscopy

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein -
islet number and veinlet termination number were carried out on epidermal strips

Preliminary Screening of Phytochemicals

The preliminary phytochemical studies were performed for testing the different chemical groups present the drugs 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. The chemical group test were performed and the result are shown in tables. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them.

Alkaliod

Test for alkaloids are following:

Dragendroff's test

Dissolve a few mg of alcoholic extract of the in 5 ml of distilled water, add 2 M hydrochloric acid until an acid reaction occurs, then add 1 ml of Dragendroff's reagents, *orange or orange-red ppt is produced immediately.*

Hager's test

To 1 ml of alcoholic extract of the drug taken in test tube, add a few drops of Hager's reagent. Formation of yellow ppt confirms the presence of alkaloids.

Wagner's test

Acidify 1 ml of alcoholic extract of the drug with1.5% v/v of hydrochloric acid and add a few drops of Wagner's reagent. A yellow or brown ppt is formed.

Mayer's reagent

Add a few drops of mayer's reagent to 1 ml of alcoholic extracts of the drug. White or pale yellow ppt. is forme

Carbohydrates

Test for crbohydrates are following:

Anthrone test

To 2 ml of anthrone test solution, add 0.5 ml of alcoholic extracts of the drug. A green or blue color indicates the presence of carbohydrates.

Benedict's test

To 0.5 ml of alcoholic extracts of the drug add 5 ml of Benedict's solution and boil for 5 mins. Formation of a brick red coloured ppt is due to presence of carbohydrates.

Fehling's test

To 2 ml of alcoholic extracts of the drug add 1 ml of the mixture of equal parts of fehling's solution 'A' and 'B' then boil the contents of the test tube for few mins. A red or brick red ppt is formed.

Molisch's test

In test tube containing 2 ml of alcoholic extracts of the drug add 2 drops of a freshly prepared 20% alcoholic solution of β napthol mix poured 2 ml of concsulphuric acid so as to form a layer below the mixture. Carbohydrates, if present, produce a red- violet ring, which disappears on the addition of an excess of alkali solution.

Flavonoids

Test for flavonoids are following:

Shinoda's test

In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dilute hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

Triterpenoids

Test for flavonoids are following:

Liebermann-Burchard's test

Add 2 ml of acetic anhydride soloution to1 ml of alcoholic extracts drug in chloroform followed by 1 ml of con sulphuric acid.A violet color coloured ring is formed shows the presence.

Saponins

In a test tube containing about 5 ml of an alcoholic extracts of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mins. Honeycomb like froth is formed.

Steroids

Test for steroids are following:

Liebermann-Burchard's test

Add 2 ml of acetic anhydride soloution to 1 ml of alcoholic extracts of the drug in chloroform followed by 1 ml of con sulphuric acid. A greenish colour is developed which turns to blue.

Salkowaski reaction

Add 1ml of conc. Sulphuricacid to 2 ml of alcoholic extracts of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer.

Tannins

Test for tanins are following:

To 1-2 ml of plant alcoholic extracts extract, add a few drops of 5% $FeCl_3$ solution was added. A green colour indicates the presence of gallotannins while brown colour tannins.

Starch

Test for starch are following:

Dissolve 0.015g of iodine and 0.075g of potassium Iodide in 5 ml of distilled water and add 2-3 ml of an alcoholic extracts of drug. A blue colour is produced.

Extraction & Characterization of Volatile and Non-Polar Component of *Conyza bonariensis*

Plant material

Plant *Conyza bonariensis* was collected from Kheri Bawli Delhi India. The plants were identified by Prof. Dr.Anju Pal, Horticulture department, panthnagar university, Panthnagar, Uttarnchal, India

Extraction and Isolation of Oils

The aerial parts of fresh plants were subjected to hydrodistillation in a Clevenger type apparatus (1 kg each) for 3 h. The distillate was saturated with NaCl and the oil was extracted with *n*-hexane and dichloromethane. The solvent phase was then dried over anhydrous Na_2SO_4

and then the solvent distilled off at 35ºC under vacuum using rotary vacuum evaporator (Buchi, Switzerland). The oil yield of plant material was 0.063% (v/w, fresh wt basis). The oil samples were stored at -20ºC until analyzed.

Instrumentation and GC Conditions

A PerkinElmer Autosystem XL gas chromatograph with flame ionization detector (GC-FID) was used, system fitted with a bonded; poly (5% diphenyl/95% dimethylsiloxane), column, EQUITY–5 (60 m x 0.32 mm, film thickness 0.25 µm, SUPELCO, USA). The column temperature ranged from70-250ºC, at 3ºC/min and 250-320ºC, at 6ºC/min, with a final hold time of 5 min, using H_2 as carrier gas at 10 psiconstant pressure, a split ratio of 1:50, an injection size of 0.03 µL neat, and injector and detector (FID)temperatures of 280ºC and 300ºC, respectively.

GC/MS utilized a PerkinElmer Autosystem XL gas chromatograph interfaced with a Turbomass Quadrupole Massspectrometer detector (GC-MS) fitted with a bonded; poly(5% diphenyl/95% dimethylsiloxane), column, fused silica capillary column EQUITY–5 (60 m x 0.32 mm, film thickness 0.25 µm, SUPELCO, USA). The column temperature of 70ºC-300ºC was programmed at a rate of 3.0ºC /min, with a hold time of 10 min. The oven temperature program was the same in as in GC while the injector temperature was 270°C, transfer line and ion source temperatures were 300°C, injection size 0.03µL neat, split ratio 1:50 using He as carrier gas at 10 psi constant pressure. MS were taken at 70 eV with a mass range of m/z 40-450. Characterization was achieved on the basis of retention time, Kovats Index, literature reported retention index. Using a homologous series of *n*-alkanes (C_8-C_{25} hydrocarbons, Polyscience Corp. Niles IL), co-injection with standards (Sigma Aldrich), mass spectra library search, and by comparing with the mass spectral literature data. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

PHARMACOLOGICAL ACTIVITY

Antibacterial Assay

Antibacterial assay was performed by agar well diffusion method [Atta-ur- Rehman *et al*,2001]. Methanolic extract and different portions dissolved in various organic solvent wereused in amount of three mg/ml of DMSO and pure compounds were used in dose of1 mg/ml. Antibacterial activity was carried out against various human pathogens

including*Escherichia coli* (ATCC 25922), *Bacillus subtilus* (ATCC 6633), *Shigellaflexenari* (clinicalisolate), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa (ATCC* 27853)*and Salmonella typhi* (ATCC 19430).In this bio assay, three types of media are required viz. solid medium (nutrient agar), semisolid medium (soft agar) and liquid medium (nutrient broth).

Solid medium (Nutrient agar)

Nutrient agar 28 g Distilled water 1 L (q.s) Nutrient agar was dissolved in distilled water and volume was made up to 1 liter. It was then placed in auto clave at 121 0C for 15 minutes. Media was then chilled to 40 0C and poured in sterile Petri dishes and media was then left to solidify at Room temperature.

RESULTS AND DISCUSSION

Macroscopic Characters of *Conyza bonariensis*

Stems

Erect perennial, rough branching extensively at the base of the plant with tapered leaves covered in stiff hairs, 20-75 cm in height. Erect stems with stiff hairs, branching extensively at the base, decreasing upwards and stems can be 20-75 cm in height.

Leaves

Narrow lanceolate leaves are grey to green in colour, measuring 2-6 cm in length, coarsely toothed and covered in fine hairs. Upper leaves are smaller and linear.

Flowers

Flowers are numerous on poorly arranged pyramidal panicles (much branched inflorescence). The capitulum (flower head) is greater than 2 mm in diameter and looks in fact like a flower bud. Flowers occur at the ends of the branches. Surrounding each flower are involucral (bell-shaped leaf-like) bracts 3-5 mm in length, the inside of each bract is white sometimes tinged purple or red. Flowering is in spring-autumn.

Fruit

The cypsela (fruit) is a linear shaped seed approximately 1.5 mm long, straw colored, covered in hairs with 16-20 noticeably longer at the top that are white or pink

Table1. Quantative Leaf Microscopy of *Conyza bonariensis*

. **Parameter**	**Range**	**Mean***
Palisade Ratio	06-10	9.85 ± 0.35
Stomatal Number Upper surface	0	0
Stomatal Number Lower surface	11-16	12.31 ± 4.81
Stomatal Index Upper surface	0	0
Stomatal Index Lower surface	11.47-11.42	10.68 ± 0.22
Vein islet number	9-11	10.64 ± 0.42
Veinlet Termination Number	5-9	18.62 ± 0.29

*** Mean value of 10 counts**

Table2. Phytochemical Screening of *Conyza bonariensis*

(-) No presence, (+) Less presence, (++) Moderate Presence, (+++) High presence, CCR: Crude powder, CPE:

Phytochemical Tests	***Conyza bonariensis* extracts**			
Active constituents	**CCR**	**CPE**	**CAC**	**CME**
Alkalodids	-	-	-	-
Saponins	+	+++	+++	++
Tannins	+	++	++	++
Steroids	+	++	++	+++
Cardiac Glycosides	+	++	+++	+++
Resins	+	+++	++	++
Starch	+++	++	++	+
Triterpenoids	+	++	+++	+++
Steroids	+++	++	+++	+

Petroleum ether extract, CAC: Acetone extract, CME: Methanol Extract, Common in CPE and CME. the Constituents can be further isolated and purified to find its potency for biological activities. [C= *Conyza bonariensis*]

VOLATILE AND NON-POLAR COMPONENT OF *C. BONARIENSIS*

The essential oil obtained by steam distillation was anlaysied by GC-FID and GC-MS. The compounds were charaterised by comparision with NIST-Wiley library of mass as well as on the basis of kovate indices. The compound characterized are summarized in table, Trans

ocimene, α- Trans-β-Farnesene, α-Sesquiphellandrene were the major components in 46.3%, 37.8.2 %, and 9.8respectively in (Table3).

Table 3. Chemical Composition of *Conyza bonariensis* and flowering plant oil.

S.No	Compound name*	RI	LRI	% Content in Aerial part	Mode of identification
1	**Trans ocimene**	1051	1463	46.3	MS
2	**α- Pinene**	930	935	0.2	RI
3	**Sabinene**	975	976	0.1	RI
4	**β-Myrcene**	987	989	4.3	MS
5	**Trans-β-Farnesene**	1473	1018	37.8	RI
6	**Nerolidol**	1578	1026	2.8	MS
7	**Germacrene-D**	1498	1031	2.3	MS
8	**α-Sesquiphellandrene**	1541	1043	9.8	MS
9	**Limonene**	1030	1383	5.1	MS
10	**β-Caryophyllene**	1426	1429	9.3	MS

RI on Equity-5 columns using a homologous series of n-alkanes (C9-C28 hydrocarbons, Polyscience Corp. Niles IL); LRI-RI reported in literature MS-mass spectrum, STD=Sigma Standard, t=trace <0.1%, * tentatively identified.

Table 4. Antibacterial assay of *Conyza bonariensis*

Table Antibacterial assay of *C. BONARIENSIS*													
S.No.	Sample	E. coli		B.Subtilus		S.flexnari		S.aureus		P.aeruginosa		S.typhi	
		1	2	1	2	1	2	1	2	1	2	1	2
1	CB-1	12	58	9	39	15	53	-	-	-	-	-	-

2	CB-2	16	58	-	-	-	-	-	-	-	-	-	-
3	CB-3	-	-	14	52	18	57	-	-	-	-	-	-
4	CB-4	19	70	15	56	-	-	-	-	-	-	-	-
5	CB-5	-	-	19	70	-	-	11	40	12	-	-	-
6	CB-6	-	-	-	-	11	52	-	-	10	39	-	-
7	STD	24	-	23	-	28	-	27	-	20	-	26	-

E. coli:Escherichia coli **S. aureus: Staphylococcus aureus**

B. subtilus:Bacillus subtilus **P. aeruginosa: Pseudomonas aeruginosa**

S. flexenari: Shigella flexenari **S. typhi : Salmonella typhi**

CB-1: Crude Methanolic extract **CB-5: n-BuOH fraction**

CB-2: n-hexane fraction **CB-6: H2O fraction**

CB-3: $CHCl_3$ fraction **STD: Imipenum.**

CB-4: EtOAc fraction

1) Zone of inhibition (mm) 2) Percent inhibition to standard drug

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