JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Pharmacognostical and phytochemical studies on *Caesalpinia bonduc* (L.) Roxb.

Suresh kumar Jebamalai and Nirmala Ramachandran
PG and Research Department of Botany, Government Arts College
(Affiliated by Thiruvalluvar University, Vellore),
Tiruvannamalai, Tamil nadu, India.

Abstracts

The selected medicinal plant *Caesalpina bonduc (L.)* Roxb. is medically valuable plants which is used to cure diseases. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in all traditional system of medicines. Pods contain phytochemical compounds were screened in qualitative manner and quantitatively estimated flavonoids, terpenoids, steroids, fixed oil and reducing sugar. Among the screening of phytochemical compounds alkaloids, protein, volatile oil and quinine are absent.

Key words: *Caesalpina bonduc*, pharmacognosy, phytochemistry,

INTRODUCTION

Medicinal plants as potential source of therapeutic aids has attained a significant role in health system all over the world for both humans and animals not only in diseased condition but also as potential material for maintaining proper health. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders.

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care[1]. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available [2].

Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (willow bark) digoxin (from foxglove) quinine (from cinchona bark) and morphine (from the opium poppy)[3].

The selected medicinal plant *C.bonduc (L.)* Roxb. is medically valuable plants which containing all parts of plants used as medicinal material for cure diseases. "Bonducella" the name of the species is derived

from the Arabic word "Bonduce" meaning a "little ball" which indicated the globular shape of the seed [4]. The seeds are grey colored and resemble eyeballs, which explains the Sanskrit name kuberakshi, meaning eyes of kubera, the Hindu God of wealth[5]. The plant was much confused with *C.bonducella* (Syn. *C. bonduc*) and was described under the same[6,7].

All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in traditional system of medicine[8]. The plant has been reported to possess anxiolytic, antinociceptive, antidiarrhoeal and antifilarial activities. Each and every part of the plant is claimed to possess some therapeutic properties, but seed kernel alone has been systematically studied so far. With the above background, the present research work is focused on ascertaining by pharmacognostical and qualitative and quantitave screening studies of *Caesalpinia bonduc* (L.) Roxb.

MATERIALS AND METHODS

Collection of plants

The plants *Caesalpinia bonduc* (L.) Roxb. was collected from Ginjee, Vilupuram District, Tamilnadu, India and the collected plants were carefully examined and identified with the help of Regional Floras [9,10,11].

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia[12]. The dried materials were subjected to methanol and aqueous extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C).

Qualitative phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* (1995) [13]. Alkaloids, carbohydrates, tannins & phenols, flavonoids, gums & mucilage's, phytosterol, proteins, fixed oils & fats, volatile oil and saponins were qualitatively analyzed.

Carbohydrates

300 mg of ethanol and aqueous extracts were dissolved separately in distilled water and filtered. The filtrate was boiled with Fehling's and with Benedict's solution. Formation of brick red precipitate in Fehling's and Benedict's solution is the positive result for reducing sugars and non-reducing sugars, respectively.

Test for carbohydrates:

A small quantity of extracts are dissolved separately in 5 ml of distilled water and filtered. The filtrate is subjected to Molisch's test to detect the presence of carbohydrates.

Molisch's test: Filtrate is treated with 2 – 3 drops of 1 % alcoholic naphthol solution and 2 ml of concentrated sulphuric acid is added along the sides of the test tube. The formation of purple colour showed the presence of carbohydrates.

Alkaloids

The extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents such as Mayer, Dragendroff's, Hager's and Wagner's reagent superlatively. Appearance of cream, orange, brown, yellow and reddish brown precipitates in response to the above reagents respectively indicate the presence of alkaloids.

Test for alkaloids:

A small portion of the extracts are stirred with a few drops of dilute hydrochloric acid and filtered and the filtrate is used for following tests.

Dragendroff's reagent: An orange precipitate obtained exhibits the presence of alkaloids.

Hager's reagent: A yellow precipitate formed it indicates the presence of alkaloids.

Wagner's reagent: A brown precipitate obtained shows the presence of alkaloids.

Steroids

Liberman Burchard test: 1 g of the extract is dissolved in few drops of dry acetic acid. 3 ml of acetic anhydride is added followed by few drops of conc. sulphuric acid. The formation of green colour provided the presence of sterols.

Tannins and Phenols

Small quantities of ethanol and aqueous extracts were dissolved in water and to that ferric chloride solution (5%) or gelatin solution (1%) or lead acetate solution (10%) was added. Appearance of blue colour with ferric chloride (or) precipitation with other reagent indicates the presence of tannins and phenols.

Saponins

1ml of each extract was dissolved separately in 20ml of water and shacked in graduated cylinder for 15 minutes. Formations of 1cm layer of foam indicate the presence of saponins.

Test for saponins:

The extracts are diluted with 20 ml of distilled water and they are agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Fixed oils and fat

- All quantity of the various extracts is separately pressed between two filter papers. An oily stain obtained shows the presence of fixed oils.
- Few drops of 0.5 N alcoholic potassium hydroxide is added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture is heated on a water bath for 1 – 2 hours. The formation of yellow coloration indicates the presence of fixed oils.
- **Gums and Mucilage**

About 10ml of extract was slowly added to 25 ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gums and mucilage.

Proteins and Free amino acids

Small quantities of various extracts are dissolved in a few ml of water and treated with

Ninhydrin reagent: A purple colouration obtained shows the presence of amino acids.

Biuret reagent: A violet colouration obtained shows the presence of proteins.

Millon's reagent: A yellow precipitate formed shows the presence of aromatic aminoacids.

Flavonoids

The extract was mixed with few ml of alcohol. It was heated with magnesium and then conc. HCl was added under cooling. Appearance of pink colour indicates the presence of flavonoids. The extract with few ml of aqueous NaOH, appearance of yellow colour and changes to colorless with HCl indicate the presence of flavonoids.

Volatile oils

For the detection of volatile oil, 50g of powdered plant material were taken in a volatile oil estimation apparatus and subject it to hydro distillation. Collect the distillate in the graduated tube of the assembly in which the aqueous portion is automatically separated from the volatile oil. If it is present in the drug, it returned back to the distillation flask.

Glycosides:

Another portion of the extract is hydrolysed with hydrochloric acid for few hours in a water bath and the hydrolysate is subjected to Legal's and Borntrager's tests to detect the presence of different glycosides.

Legal's test: To the hydrolysate, 1 ml pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. The formation of purple colour indicates the presence of glycosides.

Borntrager's test: Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. The formation of yellow colour shows the presence of glycosides.

Lipids

Lipids may be detected without alteration by spraying plates with fluorescent dyes.

Reducing sugar

Estimation of reducing sugar was done by the method of Miller (1972) [14]. The amount of reducing sugar was calculated using standard curve prepared from glucose. The quantity of reducing sugar was expressed as mg/g fresh weight of tissue.

Terpenoids (Salkowski test)

Presence of terpenoids in various fractions was determined according to Harbone (1973) [15]. 5ml (1 mg/ml) of fraction was combined with few drops chloroform, and then 3 ml of concentrated H_2SO_4. Change of reddish brown color revealed terpenoids.

Quinone (Bio assay method)

Set up five experiments in petridishes lined with filter paper circles, with 50 seeds per dish. The filter paper in the control is soaked with water ethanol (99:1). The other four are soaked respectively with the solutions of juglone, hydroquinone, arbutine and carvone containing 4 mg/mi water- ethanol (99:1) Incubate at 26^0C for 24 - 48 hour in the light. Remove dishes and count number of seeds which have germinated. From these results and those of the control, it is possible to place the four compounds in order according to their effectiveness as inhibitors [16].

ESTIMATION OF PHYTOCONSTITUENTS

The quality refers to the intrinsic value of the drug *i.e*, the amount of medicinal principles present. The active constituents are carbohydrates, glycosides, tannins, flavonoids, phenolic compounds, alkaloids, proteins and vitamins. The biological activity of a plant is influenced by the presence of various phytoconstituents. Natural antioxidants such as Vitamin C and Vitamin E directly influence the activity. Certain phytoconstituents such as phenols, tannins, carbohydrates, proteins, Vitamin C and Vitamin E are known to act synergistically. Hence, it has been quantified in the plants extract.

Estimation of Total Phenolics by Folin –Ciocalteau Method:

(*Folin –Ciocalteau method*)[17]

Different concentration of samples are taken and made up to 3 ml with water. 0.5 ml Folin-Ciocalteau reagent is added to each tube and kept for 3 minutes, to each tube 2 ml of 20 % sodium carbonate solution is added and mixed thoroughly. The test tubes are kept in boiling for 1 min. and cooled down. The absorbance is measured at 650 nm against blank and standard graph is plotted.

Estimation of Total Tannins by Folin – Danis Method:

(*Folin-Danis reagent*) [18]

Different concentration of samples are taken and made up to 3 ml with water. 0.5 ml Folin-Danis reagent is added to each tube and kept for 3 min, to each tube 2 ml of 20% sodium carbonate solution is added and mixed thoroughly. The test tubes are kept in boiling for 1 min and cooled down. The absorbance is measured at 650 nm against blank and standard graph is plotted.

Estimation of Proteins by Folin –Ciocalteau Method:

Extraction: 20 mg of the sample is homogenized in 5 ml of phosphate buffer at p^H 7.0. The extract is centrifuged at 10000 rpm for 15 minutes and the supernatant is collected. Total protein of the extract is precipitated by adding equal volumes of 5 % ice cold TCA and centrifuged at 12000 rpm for 10 minutes.

Estimation: 0.1 ml of the sample is made up to 1 ml with distilled water. 4.5 ml of Lowry's reagent is added, shaked well and allowed to stand for 10 min. To this 0.5 ml of Folin – Ciocalteau reagent is added mixed well and kept at room temperature for 20 min. A standard solution of BSA at concentration of 20 – 100 μg of blank is treated in a similar manner and a colour developed is read at 620 nm.

Estimation of vitamin - C

DNPH method [19]

Extraction: 50 mg of the powdered sample is mixed with 6 ml of 5 % TCA. The contents are shaken well and centrifuged to get a protein free extract of the vitamin.

Estimation: To 2 ml of the protein free vitamin extract, 1 drop of the indophenol reagent is added then 0.5 ml DNPH- Thio urea mix reagent is added and the mixer is incubated at 60° C for 1 hour. The tubes are then cooled in an ice water bath and 2.5 ml of 85 % sulphuric acid is added to each tube. Red colour is developed and measured at 540 nm. After 30 minutes a standard graph is prepared by taking standard ascorbic acid at a concentration 100 μg/ml.

Estimation of vitamain - E

Dipyridyl method.

Extraction: 100 mg of the sample is taken and homogenized in 10 ml of acetone and extracted. The acetone is concentrated and mixed well with 10 ml of petroleum ether and 10 ml of water in a separating funnel. The upper organic layer is separated and 10 ml of ethanol and 5 ml of 1N potassium hydroxide is added and saponified again.10 ml of petroleum ether and water is added to the saponified solution, shaken well in a separating funnel. The upper organic layer is collected and concentrated. The concentrated is dissolved in 1 ml of ethanol and used for estimation.

Estimation: 0.2 ml of the extract is made up to 1 ml with ethanol. 1 ml ∝,∝- dipyridyl reagent is added followed by 0.5 ml of ferric chloride solution. The colour is read exactly after 15 min. at 520 nm. Simultaneously a standard graph is prepared and the amount of Tocopherol is calculated.

Method of estimation of saponins

5 gm. of the sample dissolved in 50 ml. of 90 % v/v alcohol by refluxing on a water bath for ½ an hour. Filtered and the filter is washed thoroughly to take maximum quantity of soluble matter (refluxtion is repeated is dissolution is not complete). The alcoholic extract is concentrated to a thick paste. 50 ml. of Petroleum Ether 40^o to 60^o is added and refluxed for half an hour. The petroleum ether soluble portion is discarded by filtering through an extraction thimble. The thimble is transferred to a soxhelt extractor and refluxed with chloroform for half an hour and soluble portion is discarded. The same treatment is done with carbon tetra chloride and ethyl acetate and respective soluble portions are discarded. The residue is dissolved in 10 ml. methanol and poured drop wise into 50 ml. of acetone with constant stirring. The precipitate is collected, dried at 105^o to constant weight and weighed.

Estimation of total carbohydrates

Phenol –Sulfuric acid method.[20]

Extraction: 50 mg of sample is ground well with 2-3 ml of 5 % TCA. To the de-proteinized supernatant 10ml of 45 % ethanol is added to precipitate the polysaccharides. After setting it to stand overnight in cold, the tube

is centrifuged for 10 minutes at 4000 rpm. The dried precipitate is analyzed for total carbohydrate by dissolving in 2ml of 1N NaOH.

Estimation: 0.1ml of the sample is made up to 1ml with water. 1ml of 5% Phenol and 5ml of concentrated sulphuric acid are added. The mixture is mixed thoroughly with a glass rod. The solution is allowed to stand for 10 minutes at room temperature and its optical density is read at 490 nm in a spectrophotometer and standard graph is prepared by using different concentration of D-Glucose ranging from 10 to 100µg/ml.

Estimation of total lipid[21]

Cultures were taken and centrifuged at 7,000 xg for 10 minutes. From the pellet 20 mg was taken; homogenized in pestle and morter with extraction solvent (chloroform; Methanol 2:1 (v/v)) and filtered through filter paper. The filtrate was vortexes with sodium sulphate to remove moisture. Then it was taken in a pre-weighted bottle and kept this overnight at room temperature, in the dark. The dried extracts were reweighed and the total lipids were estimated by subtracting the initial weight from the final weight. The amount of total lipid was expressed as mg g^{-1} dry weight.

Estimation of fixed oils

For the quantitative estimation of oils, Soxhlet Apparatus with hexame as a solvent was used. The intact plant portion (explants) and callus weighing 5-10 g was taken and several siphons were run at 70^0C until all oil contents were extracted. Five consecutive transparent siphons indicated complete extraction. Finally, the hexame was evaporated and remaining oil sample was weighed.

Estimation of total resins

Resinous substances may occur alone or in combination with essential oil or gums. Resins white gums or insoluble in water, but they dissolve in ether, alcohol and other solvents [22] (www.facultyucr.edu.com.).

Estimation of Total Alkaloids

Weigh accurately about 10 gm. of the extract in a 150 ml. conical flask. Add 100 ml. of a mixture of 4 volume of solvent ether and 1 volume of alcohol. Add 5 ml. of dilute ammonia solution and shake frequently during one hour. Decant and filter the clear solution through cotton into a separator and wash the residue with further 100 ml. of ether alcohol mixture in 5 lots of 20 ml. each (Add gum Tragacanth powder to stimulate the stratification). To the total ether alcohol solution add 30 ml. of 1 N Sulphuric acid to make it acidic to litmus. Shake well and allow separating. Run off the lower layer continue the extraction first with 25 ml. and then with successive quantities each of volume of alcohol until complete extraction of the alkaloids is affected. Wash the mixed acid solution first with 10 ml. then with two successive quantities each 5 ml. of chloroform washing with each chloroform extract with the 20 ml. of acid alcohol mixture contained in another separator. Reject the chloroform transfer the acid liquid from second separator to the first separator make alkaline with dilute ammonia solution and add 5 ml. excess. Shake first with 25 ml. then with successive quantities each of 20 ml. of chloroform until complete extraction of the alkaloids is effected wash each chloroform extract with

10 ml. of water contained in the second separator and filter through cotton into a 150 ml. conical flask. Evaporate chloroform on a water bath, add 2 ml. of alcohol and continue the evaporation on the water bath, repeat the process using further 2 ml. of alcohol and dry the residue on the water bath for 5 minutes. Dry the residue at 105^{o} to constant weight and calculate the content of Total 'Alkaloids'.

Estimation of tannins

4 gm. of Extract and 50 ml. of water heated on a water bath for 30 minutes with frequent shaking. The liquid is allowed to settle and supernatant solution is filtered into 250 ml. std flask. Hot water extraction is continued till filtrate gives negative reaction for Tannins (Test with Ammo. Ferric Alum). The combined filtrate cooled and made up to 250 ml with water.

To 25 ml. of the solution, add 750 ml. of water and 25 ml of Indigo Sulphonic acid. The contents titrated against N/10 $KMnO_4$ to a Golden yellow end point. A blank is carried out simultaneously titrating 25 ml. of Indigo Sulphonic Acid. Each ml. of N/10 $KMnO_4$ = 0.004157 gm. of Tannins.

Preparation of Indigo Sulphonic Acid

1 gm. Indigo Caramine dissolved in 25 ml. of H_2SO_4. Further 25 ml. of H_2SO_4 added and diluted 1000 ml. with water. The acid solution to be poured, cautiously into water.

Physico chemical characteristics studies:

Total ash:

5g of the shade dried plant materials (Raw material) are taken in a pre –cleaned, pre- weighed silica crucible and maintained in a muffle furnace at 600 °C for 6 hours. The crucibles are then, taken out and, cooled at room temperature and weighed. The percentage of ash is obtained with reference to the air-dried sample.

$$\% \text{ of total ash} = \frac{\text{Difference}}{\text{Weight of sample taken}} \times 100$$

Water insoluble ash:

The total ash is boiled with 25 ml water and filtered through an ash less filter paper (whatman-41). It is followed by washing with hot water .The filter paper is ignited in the silica crucible, cooled and the water insoluble matter is weighed. The water soluble ash is calculated by subtracting the water insoluble matter from the total ash.

$$\% \text{ of water insoluble ash} = \frac{\text{Difference}}{\text{Weight of the sample}} \times 100$$

Acid insoluble ash:

The total ash obtained is boiled for five minutes with 25 ml of 2 M hydrochloric acid and filtered through an ash less filter paper. The filter paper is ignited in the silica crucible, cooled and acid insoluble ash is weighed.

$$\% \text{ of acid insoluble ash} = \frac{\text{Difference}}{\text{Weight of ash taken initially}} \times 100$$

Determination of alcohol soluble extractive:

About 2 g of the powder is macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs. Shake frequently during first 6 hrs and allowing standing for 18 hrs. It was filtered rapidly taking precautions against loss of alcohol and 10 ml of the filtrate is evaporated to dryness in a tared flat bottomed shallow dish. Dried at 105 °C and weighed. The percentage of alcohol soluble extractive is calculated with reference to the air dried powder.

$$\% \text{ of alcohol soluble extractive} = \frac{\text{Difference X 100 X100}}{\text{Weight of sample taken X 10}}$$

Determination of water soluble extractive:

About 1 g of the powder is macerated with 100 ml of distilled water in a closed flask for 24 hrs. Shake frequently during 6 hrs and allow standing for 18 hrs. It is filtered rapidly and 10 ml of the filtrate is evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive is calculated with reference to the air dried powder.

$$\% \text{ of water soluble extractive} = \frac{\text{Difference X 100 X 100}}{\text{Weight of sample X10}}$$

Determination of crude fibre content by dutch process:

About 2 gram of drug is weighed accurately and transferred to a porcelain dish. 50 ml of 10 % nitric acid is added and boiled for 30 seconds with constant stirring and filtered through fine mesh cotton cloth. The residue is washed with 5 ml of boiled water. The material from the cloth is collected in a porcelain dish and boiled with 50 ml of 2.5 % caustic soda.

Then the liquid is filtered by using the fine mesh cotton cloth. The residue is washed with 100 ml of boiling water. Then the fiber is collected in a dried, weighed crucible. The crucible is then placed at 105°C for 2 hours. This is then placed in a desiccator and cooled. The cooled crucibles are weighed.

Analysis of heavy metals by atomic absorption

Spectrophotometer

The prepared solutions are directly used for the determination of various elements by using flame photometry and AAS. These work was done by SARGHAM LABORATORY PVT. LTD. F2, Thiru-Vi-Ka Industrial Estate, (Phase-III Ekkattuthangal) Guindy, Chennai – 600 032.

Plant sample extraction

20gm of shade dried plant powder was soaked in 50ml of absolute alcohol for overnight and then filtered through Whatmann filter paper No 41 along with 2gm of sodium sulfate to remove the sediments and traces of water in the filtrate. The filter paper is wetted with absolute alcohol before filtering. The filtrate is

then concentrated by bubbling nitrogen gas into the solution and the volume is reduced to 1ml. The extract contains both polar and non-polar phyto –components.

RESULT

The selected medicinal plant *Caesalpinia bonduc* (L.) Roxb. which containing fruits used as drug for cure many ailments. The fruit pods, cotyledons and seed coats were analyzed independently in this study. The plant have pivotal role in the treatment of emmenogogue, febrigue, expectorant, anthelmintic and stomachic. In this study mainly concentrate the qualitative and quantitative estimation of phytochemicals, heavy metals, ash behavior, powder behavior studies and microbial count. The results were analysed and tabulated.

Pods contain phytochemical compounds were screened in qualitative manner. Which show flavonoids, terpenoids are in minimum amount. Steroids, fixed oil and reducing sugar are present. Alkaloids, protein, volatile oil and quinine are absent (Table – 1).

Seed coat exhibited phytochemical compounds were screened in qualitative manner. Which showed lipid, reducing sugar, terpenoids and carbohydrates are present. fixed oil, gums, mucilage, protein, flavonoids, volatile oils and quinine are absent (Table – 2).

Cotyledon contain phytochemical compounds were screened in qualitative manner. Which exhibited higher amount of positive results in carbohydrates, steroids, protein and reducing sugar but there is no response in saponins, flavanoids and lipids (Table – 3).

Medicinal plant of these three parts like pod, seed coat and cotyledon was quantitatively estimated of primary metabolites such as protein, lipid and carbohydrate. Proteins are highest in cotyledon and pod than seed coat. Lipid was highest in cotyledon. Carbohydrate content was very least in seed coat than pod and cotyledon (Table – 4).

Secondary metabolic compounds were quantified. Out of these compounds phenol high in pod, flavonoids were high in cotyledon and least amount of alkaloid recorded from all materials but vitamin C very low in cotyledon (Table – 5).

Our studied material powder were mixed with various concentrations of different chemical substances as the result of they exhibited, various colour in our normal vision. (Table – 6).

Flourescence behaviours of plant powder were carried out under UV light with various chemical substances with different concentrations. Various colours were appeared in this mixture (Table – 7).

Physiochemical evaluation of this plant was carried out through ash values, extractive values and crude fibre content. The plant powder content physiochemical percentage were calculated in average triplicate manner for total ash, water soluable ash, acid insoluble ash, crude fibre content, water soluble extract and ethanol soluble extract (Table – 8).

Analysis of heavy metals such as lead, cadmium, arsenic and mercury from the seed coat, cotyledon and pod. Among these metals, Lead exhibited in 3.32mg/kg of seed coat and 3.36mg/kg of pod and other compounds were below in detection level (Table – 9).

Phytochemical profile was estimated through the GS – MS method from seed coat, cotyledon and pod. Among these materials, cotyledon had the higher amount of phytochemicals than pod and seed coat (Table-10, 11 & 12).

DISCUSSION

The present study is undertaken to standardize *Caesalpinia bonduc* pharmacognostically which will help in the correct identification of the drug. Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [23]. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolic are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

Our plant *Caesalpinia bonduc* (L.). Roxb. , study materials pod, seed coat and cotyledon contain phytochemical compounds were screened in qualitative manner. Pod showed flavonoids, terpenoids are in minimum amount. Steroids, fixed oil, reducing sugar are present in this material and also alkaloids, protein, volatile oil, quinine are absent. Seed coat exhibit phytochemical compounds were screened in qualitative manner. Which show lipid, reducing sugar, terpenoids, carbohydrates are present. Fixed oil, gums, mucilage, protein, flavonoids, volatile oils and quinine are absent. Cotyledon exhibited higher amount of positive results in carbohydrates, steroids, protein and reducing sugar but there is no response in saponins, flavonoids and lipids.

Whole plant of *C.bonduc* contain all major chemical constituents such as steroidal saponin, fatty Acids, hydrocarbons, phytosterols, isoflavones, aminoacids, and phenolics. The unnamed alkaloid has been isolated from the leaves, stem, twig, and fruits of the plant. Phytochemical analysis of seeds of *C.bonduc* has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids [24].

Medicinal plant of these three parts like pod, seed coat and cotyledon was quantitatively estimated of primary metabolites such as protein, lipid and carbohydrate. Proteins are highest in cotyledon and pot than seed coat. Lipid was highest in cotyledon. Secondary metabolic compounds were quantified. Out of these compounds flavonoids are high in pod and alkaloids are least amount. In seed coat contain tannin in higher amount and vitamin – C was lower. Cotyledon shows flavonoids in maximum and vitamin-C minimum amount. Analysis for heavy metals such as lead, cadmium, arsenic and mercury were evaluated from this plant powder. Among these metals, mercury not detected. Lead exhibit in higher level in pod.

Isolation and structure elucidation of four new cassane diterpenes caesalpinolide-C, caesalpinolide-D, caesalpinolide-E and cassane furano diterpene along with other known compounds from *Caesalpinia bonduc*.

Has reputation of producing cassane furanoditerpenes [25,26,27,28,29]. Among cassane diterpenes production of the cassane butenolides or butenolide hemiketals is possibly via photo oxygenation of the furan ring and these compounds have very limited distribution [25,29,30,31]. But in this study we screened the phytoconstituents from pod, seed coat and cotyledon.

Flourescence behaviours of plant powder were carried out under UV light with various chemical substances with different concentrations. Various colours were appeared in this mixture.

Physiochemical evaluation of this plant was carried out through ash values, extractive values and crude fibre content were performed as per Indian Pharmacopoeia[32]. The extract of the powder was prepared with different polar and non – polar solvents for the study of successive extract values. Fluorscence analysis of the powdered drug was carried out with different chemical reagents in day (25 nm) and UV light (365 nm). The dry powder drug was studied on glass slide whereas the different extracts were studied by absorbing the extracts on Whatman filter paper[33]. In this study, physiochemical evaluation of this plant such as pods, seed coats and cotyledons were carryout out through ash values, extractive values and crude fibre content.

Ash Values were determined with a purpose to find out the total amount of inorganic solutes present in the medicinal plant material. It is also interesting to know about the different solubility of the components of ash. Our selected plant powder content physiochemical percentages were calculated.

Conclusion

The selected medicinal plant *Caesalpinia bonduc* (L.) Roxb. belong to family of Caesalpiniaceae, which consisting fruit was selected as a study material in the manner of pod, seed coat and cotyledon.

We studied qualitative and quantitative of phytochemicals heavy metals. The pharmacological activity of powder behavior and ash behavior were examined. The phytochemical profile was carried out through GC-MS method.

Pod exhibited positive results on flavonoids, terpenoids, steroids, fixed oil and reducing sugar. Seed coat gave positive results in lipid, reducing sugar, terpenoids and carbohydrates. Cotyledon containing carbohydrate, steroids, reducing sugar and proteins were screened through qualitative test.

The highest amount of primary metabolic compounds were estimated, proteins on pod (11.26 w/w %) and cotyledon (29.43 w/w %). The highest amount of Lipids were estimated from cotyledon (12.14 w/w %) and lowest from pod (3.122w/w%). Among the tested secondary metabolic compounds, phenol were high (9.123 w/w %) and least amount of alkaloids (0.778 w/w %) in pod. Seed coat showed tannin in higher amount (3.472 w/w %) and lower of vitamin – C (0.087 w/w %). Highest amount of flavonoids (18.24 w/w %) and lowest amount in vitamin – C (0.062 w/w %) in cotyledons.

Various colours in our normal vision exhibited by various concentrations of different chemical substances mixed with material powder. Various colours were appeared in mixture when plant powder carried out under UV light with various chemical substances with different concentrations.

Total ash, water soluble ash, acid insoluble ash, crude fibre content, water soluble extract and ethanol soluble extract were calculated by average triplicate manner of plant powder content physio-chemical percentage. Higher amount Phytocomponents in cotyledons and lower in seed coat were elucidated through GC-MS methods. Higher amount of lead estimates from pod (3.36 mg / kg).

REFERENCES

1. Kamboj V P. Herbal Medicine. Current Science 2000; 78:35-9.

2. Gupta LM, Raina R. Side effects of some medicinal plants. Current Science 1998; 75, 897-900.

3. Vickers A, Zollman C. ABC of complementary medicine: Herbal medicine. British Medical Journal 1999; 319: 1050 -1053.

4. Handa SS, Kaul MK. Herbs as medicines. Supplement to cultivation and utilization of medicinal plants. RRL, jammu-tawi; 1996. p. 727-737.

5. Jha M. Community based conservation and management of medicinal plants in India, The Indian Forester. 2003; 129: 187-197.

6. Shah GL. Flora of Gujarat State; 1978. Part 1, p. 264.

7. Ram P, Rastogi, Mehrotra. Compendium of Indian medicinal plants, CDRI, Lucknow And PID CSIR, New Delhi: 1991; 1: 67.

8. Kirtikar KR, Basu BD. **In**: Blatter, E, Caius JF, Mhaskar KS. (Eds.), Indian Medicinal Plants, vol. III. Lalit Mohan Basu, Allahabad, India: 1993.

9. Gamble JS Flora of the Presidency of Madras (vol. 1). London: forgotten books; 1915; 1: 278 - 279.

10. Henry AN, Kumari GR, Chitra V. Flora of Tamil Nadu India. Series 1: Analysis. Vol. 2, Botanical Survey of India; Coimbatore; 1987.

11. Mathew KM. The flora of the Tamil Nadu Carnatic, vol. I –III. The Rapinet Herbarium, St. Joseph's College, Trichirapalli. India; 1983.

12. Anonymous. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. The Controller of Publications, Civil Lines, Delhi, 1996; Vol. I & II.

13. Kokate CK, Khandelwal KR, Pawar AP, Gohale SB. Practical Pharmacognosy. Nirali Prakashan; Pune;1995. 3rd edn. p. 137-139.

14. Miller LG. Herbal Medicinals: Selected clinical considerations focusing on known or potential drug-herb interactions. Archives of International Medicine. 1998; 158: 2200–11.

15. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis, 1973. p. 522-528.

16. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed. Chapman and Hall, Londan;1984. p. 288.

17. Bray HG, Thorpe WV. Methadology of biochemical analysis. 1954; 1: 27-52.

18. AOAC. Official methods of analysis, 13th Ed. Association of official analytical chemists, Washington D.C; 1980; p. 376-384.

19. Sarojini Y, Nittala SS. Vitamin C content of some macroalgae of Visakhapatnam, East coast of India. Indian J, Mar, Sci 28, 1999. p. 408-12.

20. Dubois M, Giles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 1956; 28: 350-356.

21. Sato M, Fujiwara S, Tsuchiya H, Fujii T, Iiunuma M, Tosa H, Ohkaw Y. Flavones with antibacterial activity against cariogenic bacteria. Ethnopharmacology 1996; 54: 171-176.

22. www.facultyucr.edu.com.

23. Exarchou V, Nenadis N, Tsimidou M, Gerothae.nassis IP, Troganis A, Boskou, D. Antioxidant activities and phenolic composition of extract from Greek oregano, Greek. sag and summer savory . Journal of Agriculture and Food chemistry 2002; 50 (19): 5294-5299.

24. Gaur RL, Sahoo MK, Dixit S, Fatma N, Rastogi S, Kulshreshtha DK, Chatterjee RK. Antifilarial activity of *Caesalpinia bonducella* against experimental filarial infections. Indian Journal of Medical Research 2008; 128: 65-70.

25. Kinoshita T. Chemical studies on the philippine crude drug calumbibit (seeds of *Caesalpinia bonduc*): the isolation of new cassane diterpenes fused with alpha,beta-butenolide. Chemical & Pharmaceutical Bulletin 2000; 48(9): 1375-1377.

26. Kalauni, Surya & Awale, Suresh & Tezuka, Yasuhiro & Banskota, Arjun & Linn, Thein & Kadota, Shigetoshi. Methyl Migrated Cassane-Type Furanoditerpenes of Caesalpinia crista from Myanmar. Chemical & pharmaceutical bulletin 2005; 53. 1300-4.

27. Lyder DL, Sonia R, Peter, Winston F, Tinto, Suzanne M, Bissada, Stewart Mclean, william f. Reynolds. Minor Cassane Diterpenoids of *Caesalpiniabonduc.* J. Nat. Prod 1998; 61, 12, 1462–1465.

28. Linn TZ, Awale S, Tezuka Y, Banskota A, Kalauni SK, Attamimi F, Ueda JY, Asih PB, Syafruddin D, Tanaka K, Kadota S. Cassane and norcassane type diterpenes from *Caesalpinia crista* of Indonesia and their antimalarial activity against the growth of *Plasmodium falciparum*. J. Nat. Products, 2005; 68(5): 706-10.

29. Lyder, DL, Tinto, WF, Bissada SM. Caesalpinin B, a rearranged Cassane diterpenoid of *Caesalpinia bonduc*. Heterocycles. 1998;48, 1465–1469.

30. Daniel Torres-Mendoza, Luis David Ureña González, Eduardo Ortega-Barría, Phyllis D. Coley, Thomas A. Kursar, Todd L. Capson, Kerry McPhail, Luis Cubilla-Rios. Novel Cassane and Cleistanthane Diterpenes from *Myrospermum frutescens*: Absolute Stereochemistry of the Cassane Diterpene Series. J. Nat. Prod 2004; 67, 10, 1711–1715.

31. Roach, John & Andrzejewski, Denis & Gay, Martha & Nortrup, David & Musser, Steven. Rugged LC-MS/MS survey analysis for acrylamide in food. Journal of agricultural and food chemistry 2004; 51. 7547-54.

32. Anonymous. Indian Herbal Pharmacopoeia. Indian Drug Manufactures Association and Regional Research Laboratory; 1966.

33. Kokoski CJ, Kokosk, RJ, Sharma M. Flourescence of powdered vegetable drugs and ultraviolet radiation. Journal of America Pharmaceutical Association 1958; 47: 715-717.