

# Phytochemical Analysis of Stem Bark and Root Bark of *Zizyphus Mauritiana*

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## Abstract

*Zizyphus mauritiana* is a plant which is commonly found in the temperate regions. In *Ayurveda*, it is considered to be a medicinal plant with immense importance. Right from the leaves to roots, all parts of this plant are reported to possess good medicinal value. For a medicinal plant, the actual wealth is the phytochemicals which it contains. Stem and root barks of medicinal plants have been given special importance in *Ayurveda*. Here, stem and root barks of *Zizyphus mauritiana* are successively extracted in various solvents in the increasing order of polarity. Solvents in the extracts are evaporated and phytochemical analysis of the dried powder is performed. It has been observed that almost all important phytochemicals are present in one or the other extracts in more or less quantity. From phytochemical screening, it is proved that *Zizyphus mauritiana* is really the storehouse of phytochemicals.

## 1. Introduction

India is considered to be the origin of *Ayurveda*. As far as biodiversity is concerned, India is amongst the topmost countries. Forests rich in medicinal plants are the identity of India. Most of the medicinal properties of plants are due to the phytochemicals, which they contain. Phytochemicals are the non-nutritive secondary metabolites that have defensive or disease preventive properties (Tan *et al.*, 2010). Plants synthesize these chemicals to protect themselves. e.g. plants are reported to produce phytoalexins in response to attack by bacteria and fungi (Hammerschmidt, 1999). Phytochemicals are actually not required for the immediate survival of the plants but are synthesized by the plants to increase their own fitness for proper survival. These compounds allow the plants to interact with their surrounding environment including pathogens, insects and herbivores and also contribute to the plant's color, aroma and flavor. They also protect the plants from environmental hazards such as pollution, stress, draught and UV exposure. Thus phytochemicals are one of the means of adaptation (Gibson *et al.*, 1998). Amongst plants there is competition for better

survival and thus they keep on modifying their phytochemicals and try to be better with each passing generation (Kennedy *et al.*, 2011). Current research revealed that they can also protect humans and other animals against diseases (Russo *et al.*, 2012). There are thousands of well known phytochemicals and many thousands are under investigations (Meagher and Thomson, 1999). Every plant in nature contains few or more phytochemicals. Some of them might be toxins but most are found to be useful in some or other diseases. Phytochemicals are found to be present in all parts of plants including leaves, fruits, seeds, flowers, stems and roots, but stem barks and root barks are seen to possess a wide variety of phytochemicals. That might be the reason why they are important ingredients of many *Ayurvedic rasayanas* as well as Unani medicines.

Today's shining world of phytomedicines was not so, right from the beginning. It has gone through many ups and down and a lot of struggle. Herbal preparations were in use from centuries but isolation of first few active principle alkaloids like morphine and quinine in the early 19<sup>th</sup> century started a new era in the science of medicinal plants and thus modern medicinal plant research came into existence. But, tremendous development in synthetic pharmaceutical chemistry and microbial fermentation after 1945 put the plant derived drugs out of picture. Science of phytomedicines almost went into extinction during the last decade of the 20<sup>th</sup> century due to the use of more powerful and potent synthetic drugs. As side effects of these drugs started showing their effects, people turned their minds towards phytomedicines and medicinal plants earned their values again. Though the effects of natural remedies are slower, the results are many times much better on the long run especially in chronic diseases (Akunyili, 2003). Over the last decade, however, interest in medicinal plants and their compounds has grown steadily (Hamburger and Hostettmann, 1991). Utilization of medicinal plants in Western Europe during this period has almost doubled. Awareness regarding natural products, the efficacy of phytochemical preparations like *Vicco turmeric*, *Safi*, *Drakshasav* etc. and increased interest of major pharmaceutical companies

(*Vicco, Himalaya, Baidyanath* etc.) in variety of medicinal plants as sources for new compounds has been the main reasons for this renewal of interest. With the development of newer and better isolation techniques and revolution in analytical chemistry, scientists began to extract chemical products from medicinal plants. As various active principles in medicinal plants got identified and isolated, plant based prescriptions began to be substituted more and more with pure substances, which were as effective as the synthetic ones and easier to prescribe and administer (Harvey, 2000). Today, *Patanjali Yog Pitha* is one of the largest seller of plant derived medicinal compounds and has taken lots of efforts to teach people about the importance of Indian traditional system of medication not only in India but also throughout the world.

Chemically phytochemicals are classified as Alkaloids, Anthocyanins, Anthracene glycosides, Anthraquinones, Aucubins & Iridoids, Carbohydrates, Cardiac glycosides, Carotenoids, Coumarins, Emodins, Flavonoids, Polyuronoids, Reducing sugars, Saponins, Steroids, Tannins and Triterpenoids (Hahn, 1998). Different phytochemicals with almost all types of medicinal properties including anti-spasmodics, anti-inflammatory, anti-viral, anti-cancer, anti-fungal, anti-bacterial, anti-oxidant, anti-malarial, anti-ulcer, anti-hypertensive, anti-depressive, hypocholesterolemic, immunomodulatory, clot dissolving, detoxifying and many others are reported (Mamta *et al.*, 2013).

*Zizyphus mauritiana* is referred as the storehouse of phytochemicals and is considered as a plant of immense importance in *Ayurveda*. Fruits of the plant are eaten up in India quite pleasantly. But, not a single report of any side effects has been recorded.

Extraction is the separation of medicinally active portions of plants using selective solvents. For extraction purpose, soxhlet assembly is generally used in which phytochemicals are separated from dried powder of plant parts in the increasing order of polarity. Compounds with particular polarity get dissolved in the solvent of same polarity by either nonpolar-nonpolar interaction or polar-polar interaction. During successive extraction, care has to be taken to dry the powder between two successive

extractions so that to evaporate the previous solvent completely. The extracts are dried and the powder can be used to check out the activity. Phytochemical screening of the extracts can be done by dissolving them in 0.1% DMSO in phosphate buffered saline (PBS).

## 2. Materials and methods

### 2.1. Reagents and chemicals

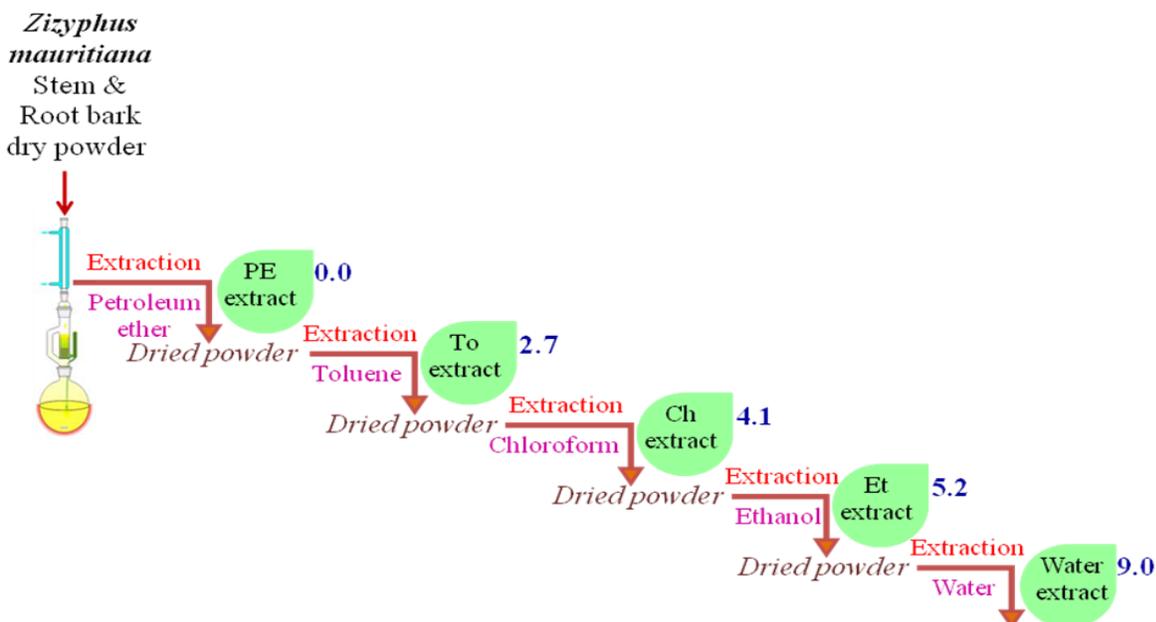
All the solvents (Petroleum ether, Toluene, Chloroform, Ethanol) used for extraction purpose were of HPLC grade and were purchased from authorized standard companies. All other chemicals required for phytochemical screening are of analytical grade.

### 2.2. Plant materials

Stem bark & root bark of plants were collected from forest region of Gadchiroli district of Maharashtra (India) in May 2009 (summer). While taking out barks complete care was taken to prevent any injury to plants. Authentication was done at University Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur and voucher specimens were deposited in herbarium. Botanical name of the plant was identified to be *Zizyphus mauritiana* (Family Rhamnaceae) and relevant voucher specimen number was 9483 (Date: 09/10/2009).

### 2.3. Preparation of extracts

Stem and root bark of *Zizyphus mauritiana* were carefully cleaned with sterilized damp cloth and shed dried separately for approximately two months. Dried bark was crushed in grinder. Using soxhlet apparatus, powdered bark was extracted successively in increasing order of polarity by petroleum ether (0), toluene (2.7), chloroform (4.1), ethanol (5.2) and water (9). For extraction, 250g of dried powder was used. Solvents from the extracts were evaporated by using rotary vacuum evaporator (Superfit DB3135S) while aqueous extracts were freeze-dried and stored at -20°C as this low temperature reduces degradation of the bioactive natural product. Extraction process by using different solvents with respect to polarity is illustrated in the given scheme.



**Fig. 1. Extraction with respect to Polarity**

#### 2.4. Phytochemical screening

Dried extracts were dissolved in 0.1% Dimethyl sulphoxide (DMSO) in PBS (phosphate buffered saline), mixed and vortexed for 1min. Supernatants obtained after centrifugation at 100g for 2min were tested for phytochemical analysis. Qualitative phytochemical analysis was performed as per the methods proposed in Trease and Evans (Evans, 2002) for almost all known phytochemicals.

#### 1. Alkaloids

A few mg of dry extract powder was taken separately in 5ml of 1.5% hydrochloric acid solution and filtered. Following tests were performed in the resulting acidic solution of extract:

**Iodine Test:** Few drops of dilute iodine solution were added in 3ml of acidic extract solution. Blue colour formation, which disappears on boiling and reappears on cooling, indicated the presence of alkaloids.

**Wagner's Test:** Few drops of Wagner's reagent (1.27g Iodine + 2g Potassium Iodide dissolved in 5ml distilled water and diluted to 100ml) were added in 2ml of acidic extract solution. Development of reddish brown precipitate gave a positive sign for alkaloids.

**Dragendorff's Test:** Few drops of Dragendorff's reagent (Solution A: 17g Bismuth subnitrate + 200g tartaric acid dissolved in 800ml distilled water; Solution B: 160g Potassium iodide in 400ml distilled water; Mix both A and B solutions in 1:1 proportion) were added in 2ml of acidic extract solution.

Formation of orange brown precipitate was due to the presence of alkaloids.

#### 2. Anthocyanins and Anthocyanidins

Formation of red color in acidic aqueous solutions of extracts at pH 3-4 indicated the presence of anthocyanins and change of color with pH modification (pH 8-9) indicated the presence of anthocyanidins.

#### 3. Anthracene Glycosides

Ethereal solutions of extracts were treated with 25% ammonium hydroxide. Development of red color indicated the presence of Anthracene glycosides.

#### 4. Anthraquinones

The dried extracts were mixed with 0.5% w/v aqueous potassium hydroxide with continuous maceration for about 1h. To the alkaline extract was added 1ml each of hydrogen peroxide, acetic acid and benzene and finally equal volume of dilute ammonia solution was added. The development of red color in the ammoniacal layer indicated the presence of anthraquinones.

#### 5. Aucubins and Iridoids

Dried extracts were treated with 5ml of 1% aqueous hydrochloric acid. After 3-6 hr, the acidic extract was treated with 1ml of Trim Hill reagent (10ml acetic acid, 1ml of 0.2% w/v copper sulphate in water and 0.5ml of concentrated hydrochloric acid) and heated on boiling water bath. The development of blue color gave an indication of the presence of aucubins

(Diterpenoids) while green or red color gave an indication of the presence of iridoids (Monoterpenoids).

### 6. Carbohydrates

Following tests were performed for the presence of sugars:

**Molish's test:** A few drops of 10% solution of 1-naphthol in alcohol were added in few ml of extract solution. One ml of conc.  $H_2SO_4$  was added along the walls of the test tube. Appearance of red-violet ring at junction of two layers indicated the presence of carbohydrates.

**Barfoed's test:** A few ml of Barfoed's reagent (13.3g of crystalline neutral copper acetate was dissolved in 120ml of 1% v/v aqueous acetic acid solution) was heated with a few ml of extract solution, presence of monosaccharides is indicated by development of red color due to cuprous oxide.

### 7. Cardiac Glycosides

For cardiac glycosides, following tests were performed:

**Kedde's test:** The dried extracts were dissolved in few drops of methanol and 2-3 drops of Kedde's reagent (1.5% 3-dinitrobenzoic acid in methanol) followed by 2 drops of 2N methanolic sodium hydroxide solution were added. Blue or violet color indicated the presence of cardiac glycosides.

**Legal test:** Powdered extracts were dissolved in pyridine, to this Sodium nitroprusside solution was added and made alkaline with NaOH solution. Pink color indicated the presence of cardiac glycosides.

### 8. Carotenoids

Concentrated hydrochloric acid and phenol were added to the solution of extracts. The development of blue/green color indicated the presence of carotenoids.

### 9. Coumarins

The dried extracts were dissolved in ether. The resulting solutions were taken in test tubes, covered with filter paper moistened with diluted NaOH solution and were placed in water bath at boiling temperature for 20-30min. The filter papers were removed and observed under UV light in UV chamber. Yellow green fluorescence indicated the presence of coumarins.

### 10. Cyanogenic glycosides

Few drops of chloroform were added, to the 2g of dried powdered extract. Sodium picrate solution (5 g Sodium carbonate, 0.5 g Picric acid, and 100 mL distilled water) was prepared and strips of filter paper

were moistened in this solution. These strips were dried in air and inserted in test tube containing the reaction mixture. Precaution was taken to ensure that the strips should not touch inner walls of test tube containing the reaction mixture. The test tube contents were warmed at 30-35 °C for half an hour. The development of red color on paper indicated the presence of cyanogenic glycosides (Das and Bhattacharjee, 1970).

### 11. Emodins

Presence of emodins was marked by Borntrager's reagent (Chhabra *et al.*, 1984). Dry extracts were dissolved in benzene, followed by addition of 25% ammonium hydroxide. The development of red color marked the presence of emodins.

### 12. Flavonoids

Presence of flavonoids was tested by Shinoda's reaction (Shinoda, 1928). The dried extracts were dissolved in ethanol. On addition of Magnesium powder and concentrated hydrochloric acid, the development of yellow/red color indicated the presence of flavonoids.

### 13. Polyoses

Extract solution were treated with 2-3 drops of concentrated sulphuric acid followed by addition of 3-4 drops of alcoholic thymol. Development of red color indicated the presence of polyoses (Chhabra *et al.*, 1984).

### 14. Polyuronoids

To a few ml of the extract solution, equal volume of hematoxylin solution was added. The formation of a persistent violet precipitate insoluble in ethanol indicated the presence of polyuronoids (Hinton, 1951).

### 15. Reducing Sugars

To the extract solutions, few drops of Fehling's solution [mix equal volumes of Fehling's solution A (copper sulphate solution) and Fehling's solution B (alkaline tartarate solution)] was added. This mixture was heated for 5min. The samples positive for reducing sugars developed brick red precipitate.

### 16. Saponins

A few ml of the extract solution was shaken vigorously for 10 seconds and allowed to stand. Development of a persistent honeycomb like froth gave an indication of the presence of saponins.

### 17. Starch

The appearance of blue color by the addition of **Lugol solution** (1g of Iodine and 2g Potassium iodide dissolved in distilled water and make up to 300ml) in solution of extract indicated the presence of starch.

### 18. Steroids

For steroids, following tests were performed:

**Salkowski reaction:** A few mg of dried powdered extract was taken in 2ml of the chloroform. To this, conc. H<sub>2</sub>SO<sub>4</sub> was added along the side walls of the test tube. The test tube was shaken for few minutes. Red color was developed in chloroform layer and lower layer of acid gave greenish yellow fluorescence. This colorization and fluorescence was due to the presence of steroids.

**Libermann-Burchard reaction:** A few mg of dried powdered extract was dissolved in chloroform. To this was added few ml of acetic anhydride and two drops of conc. H<sub>2</sub>SO<sub>4</sub> along the side walls of the test tube. Formation of transient greenish color was a positive signal for the presence of steroids.

### 19. Tannins

For tannin, following tests were performed:

**Ferric chloride test:** To 1ml solution of extract, 2-3 drops of dilute ferric chloride solution were added, development of green black color indicated the presence of tannins.

**Gelatin test:** To 1ml solution of extract, 2-3 drops of gelatin solution was added, formation of white precipitate gave positive signal for tannins.

**Lead acetate test:** To 2ml solution of extract, 2-3 drops of 10% lead acetate solution was added, formation of red precipitate indicated the presence of tannins.

### 20. Triterpenoids

For the presence of triterpenoids, **Libermann-Burchard reaction** was performed. In this, a few mg of dried powdered extract was dissolved in chloroform. To this was added few ml of acetic anhydride and two drops of conc. H<sub>2</sub>SO<sub>4</sub> along the side walls of the test tube. Formation of red/violet color indicated the presence of triterpenoids.

## 3. Results

### 3.1. Quantity of extracts obtained in each solvent

**Table 1.**

Quantity of dry extract (g) obtained from 250g **dry bark** after successive soxhlet extraction using Petroleum Ether (polarity 0), Toluene (polarity 2.7), Chloroform (polarity 4.1), Ethanol (polarity 5.2) and Water (polarity 9).

| Solvent         | Dry Powder Stem bark (g) | Dry Powder Root bark (g) |
|-----------------|--------------------------|--------------------------|
| Petroleum Ether | 1.02                     | 0.86                     |
| Toluene         | 1.35                     | 1.11                     |
| Chloroform      | 1.89                     | 1.62                     |
| Ethanol         | 6.74                     | 7.50                     |
| Water           | 3.31                     | 4.05                     |

### 3.2. Phytochemical analysis of each extract of stem bark

**Table 2.**

Phytochemical screening of the extracts of stem bark in Petroleum Ether, Toluene, Chloroform, Ethanol and Water.

| Test                            | Petroleum Ether | Toluene | Chloroform | Ethanol | Water |
|---------------------------------|-----------------|---------|------------|---------|-------|
| Alkaloids                       | ++              | ++      | ++         | ++++    | ++    |
| Anthocyanins and Anthocyanidins | -               | -       | -          | +       | +     |
| Anthracene glycosides           | -               | -       | +          | ++      | ++    |

|                              |     |    |     |     |      |
|------------------------------|-----|----|-----|-----|------|
| <b>Anthraquinones</b>        | +   | +  | ++  | -   | -    |
| <b>Aucubins and Iridoids</b> | -   | -  | -   | +   | +    |
| <b>Carbohydrates</b>         | -   | -  | -   | ++  | +++  |
| <b>Cardiac glycosides</b>    | -   | +  | +   | ++  | +++  |
| <b>Carotenoids</b>           | ++  | +  | +   | -   | -    |
| <b>Coumarins</b>             | ++  | +  | +   | -   | -    |
| <b>Cyanogenic glycosides</b> | -   | -  | +   | +   | ++   |
| <b>Emodins</b>               | -   | -  | +   | +   | -    |
| <b>Flavonoids</b>            | -   | +  | +++ | ++  | ++   |
| <b>Polyoses</b>              | -   | -  | +   | +   | ++   |
| <b>Polyuronoids</b>          | -   | -  | -   | +   | +    |
| <b>Reducing Sugars</b>       | -   | -  | -   | +   | +++  |
| <b>Saponins</b>              | +   | +  | +   | +   | +    |
| <b>Starch</b>                | +   | +  | +   | +   | -    |
| <b>Steroids</b>              | +++ | ++ | ++  | -   | -    |
| <b>Tannins</b>               | -   | -  | +   | +++ | ++++ |
| <b>Triterpenoids</b>         | -   | ++ | +   | ++  | +    |

### 3.3. Phytochemical analysis of each extract of root bark

**Table 3.**

Phytochemical screening of the extracts of root bark in Petroleum Ether, Toluene, Chloroform, Ethanol and Water.

| <b>Test</b>                            | <b>Petroleum Ether</b> | <b>Toluene</b> | <b>Chloroform</b> | <b>Ethanol</b> | <b>Water</b> |
|--|------------------------|----------------|-------------------|----------------|--------------|
| <b>Alkaloids</b>                       | +                      | +              | +                 | ++             | ++           |
| <b>Anthocyanins and Anthocyanidins</b> | -                      | -              | -                 | -              | +            |
| <b>Anthracene glycosides</b>           | -                      | -              | -                 | +              | ++           |
| <b>Anthraquinones</b>                  | ++                     | +              | -                 | -              | -            |
| <b>Aucubins and Iridoids</b>           | -                      | -              | -                 | +              | ++           |

|                              |     |    |     |    |      |
|------------------------------|-----|----|-----|----|------|
| <b>Carbohydrates</b>         | -   | -  | -   | ++ | ++   |
| <b>Cardiac glycosides</b>    | -   | +  | +   | +  | +++  |
| <b>Carotenoids</b>           | +++ | -  | -   | -  | -    |
| <b>Coumarins</b>             | ++  | ++ | -   | -  | -    |
| <b>Cyanogenic glycosides</b> | -   | -  | -   | ++ | ++   |
| <b>Emodins</b>               | -   | -  | +   | ++ | -    |
| <b>Flavonoids</b>            | -   | -  | +++ | ++ | +    |
| <b>Polyoses</b>              | -   | -  | +   | ++ | ++   |
| <b>Polyuronoids</b>          | -   | -  | -   | ++ | +    |
| <b>Reducing Sugars</b>       | -   | -  | -   | +  | +++  |
| <b>Saponins</b>              | +   | -  | +   | +  | -    |
| <b>Starch</b>                | +   | +  | +   | -  | -    |
| <b>Steroids</b>              | +++ | ++ | +   | -  | -    |
| <b>Tannins</b>               | -   | -  | +   | ++ | ++++ |
| <b>Triterpenoids</b>         | -   | +  | ++  | +  | +    |

Phytochemical screening of all the extracts of stem and root bark has shown the presence of Alkaloids, Anthocyanins, Anthracene glycosides, Anthraquinones, Aucubines & Iridoids, Carbohydrates, Cardiac glycosides, Carotenoids, Coumarins, Emodins, Flavonoids, Polyuronoids, Reducing sugars, Saponins, Starch, Steroids, Tannins and Triterpenoids. These results are specified in the Table 2 and Table 3.

#### 4. Discussion

Phytochemical screening of the extracts of stem bark and root bark of *Zizyphus mauritian* revealed the presence of various phytochemicals of which alkaloids, flavonoids and tannins were the most prominent. Stem bark had shown the presence of more amounts of alkaloids while root bark was found to be more prominent in tannins. Almost all phytochemicals are found to possess antioxidant properties and they act as scavengers of free radicals; that is Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Rice-Evans *et al.*, 1997; Jorgensen, 1999) and many of them are used for wide

range of treatments including inflammation, kidney problems, stomach disorders and many others (Zhu *et al.*, 1997). Many naturally occurring **triterpenoids** isolated from various parts of plants have been reported to be good anti-inflammatory compounds (Ismaili *et al.*, 2002). Along with anti-inflammatory activity, they have been seen to possess many other biological activities like bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anticancer and antiallergic (Patocka, 2003). **Coumarins and tannins** are known to have antibacterial activities (Soine, 1964). They are also found to be active against pathogen causing diarrhea (Choi *et al.*, 2009). Tannins specially are a group of certain phytochemicals with wide variety of properties such as antiviral, antiparasitic, anti-inflammatory and antiulcer (Bajaj, 1988; Lu, 2004; Akiyama, 2001; Kolodziej, 2005). **Alkaloids**, a wonderful group of phytocompounds have been well studied for their anti-arrhythmic, analgesic, anti-hypertensive, antipyretic, antimalarial and antitumor activities and their use in the treatment of cough and gout is very common (Robert, 1992). Variety of

**flavonoid**shavebeen reported for their antiviral, anti-inflammatory and cytotoxic activities (Chhabra *et al.*, 1984) and are very common in the treatment of capillary fragility, retinal hemorrhage, hypertension, diabetes, retinopathy, rheumatic fever and arthritis (Tripathi *et al.*, 1981). **Steroids** and **triterpenoids** are known to posses anti-inflammatory activities(Chawla *et al.*, 1987). **Saponins** are well known expectorant. They are of great importance as they have their direct impact on sex hormones, diuretic steroids, vitamin D and cardiac glycosides (Madarnas *et al.*, 1989). Saponins of medicinal plants are active in the action of tribal medicines as diuretic and remedy for cough. **Cardiac glycosides** are large group of compounds, which affect the heart muscles. These show a good strengthening effect on weaker hearts(Wang, 2008). **Carotenoids** are of nutritional value. They are the modified forms of vitamin A (Delgado-Vargas *et al.*, 2000). Vitamins are consumed as dietary supplements in the form of capsules or syrups andmedicinal plants can serve as source of vitamin A.

To make the best and judicious use of available natural wealth, a number of medicinal plants have been chemically investigated by various workers. The active principles isolated from such chemical analysis

are used as medicines. With the overall development of Indian system of medicine, there is an essential requirement for the discovery of new drugs. In order to find the new drugs and their sources, a number of plants are screened yearly for their biological activities in India.

The scientific screening and evaluation of Indian plants for their medicinal values is signalingthe whole world towards the natural treasure of new drugs in indigenous and folklore system of medicine. Phytochemical screening of plants indicates the presence of compounds which can be useful in the treatment of almost all diseases.

### 5. Conclusion

Phytochemical analysis indicates that *Zizyphus mauritiana* is the storehouse of phytochemicals. Almost all phytochemicals are found in one or the other parts of this creative plant. The various compounds isolated from this plant can serve as new drugs and can be screened for the treatment of many diseases and disorders. Not only root bark and stem bark, but also leaves, fruits and flowers are found to be rich in phytochemical content.

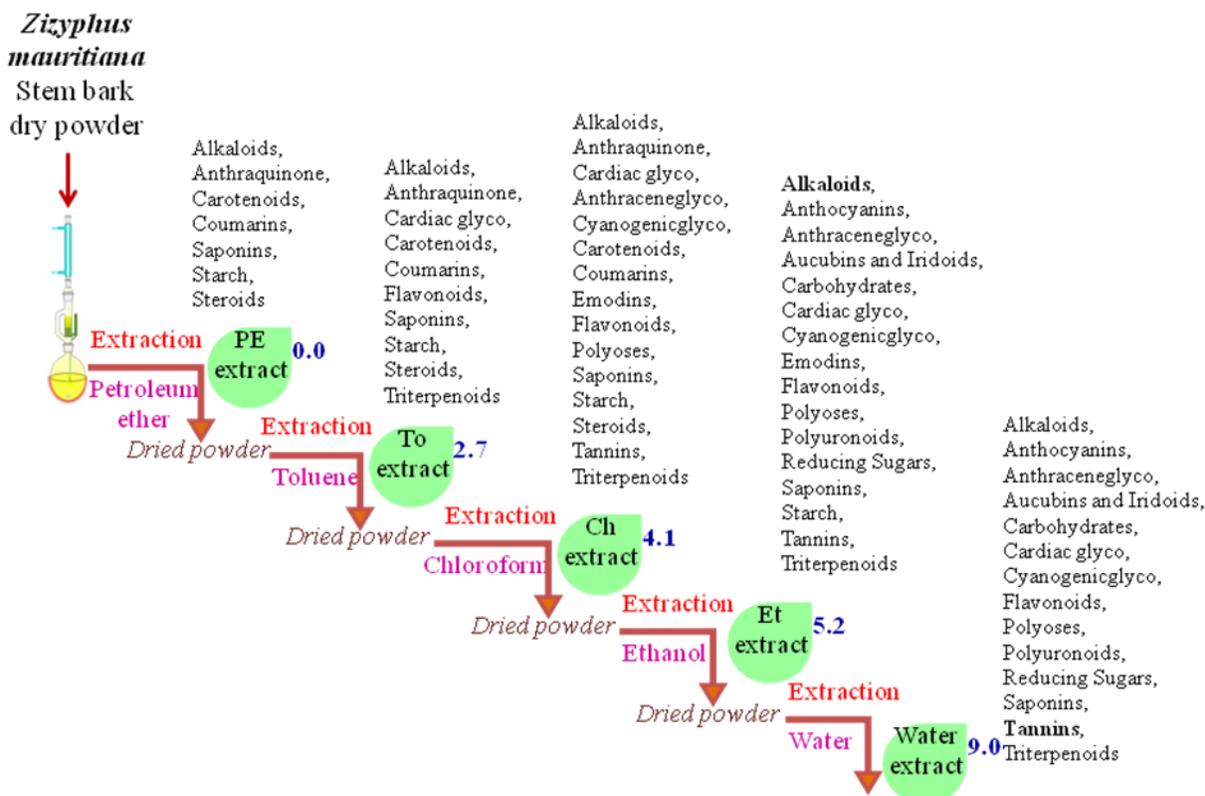
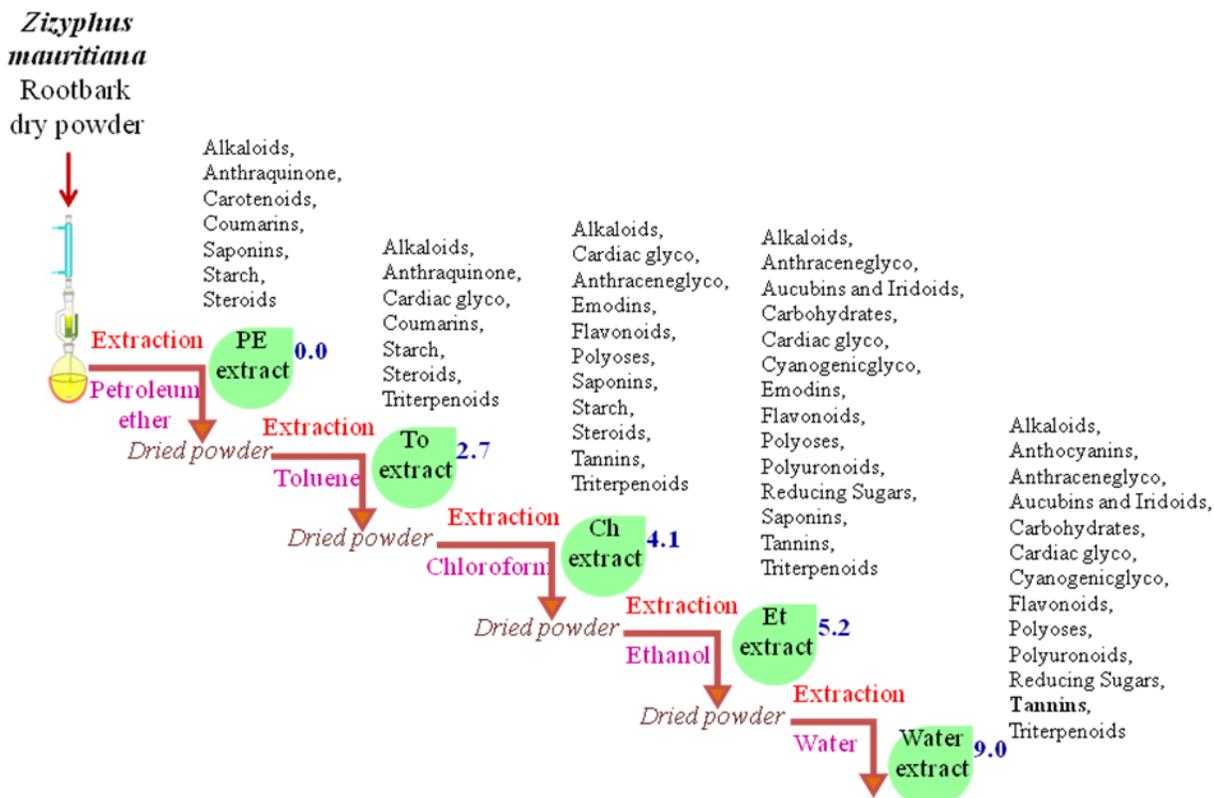


Fig. 2. Scheme showing extraction process as well as results of phytochemical analysis of the extracts of stem barks prepared in different solvents.



**Fig. 3. Scheme showing extraction process as well as results of phytochemical analysis of the extracts of root barks prepared in different solvents.**

### References

- Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. *J Antimicrob Chemothe.* 2001; 48 (4): 487–491.
- Akunyili DN. The role of regulation of medicinal plants and phytomedicine in socio-economic Development, AGM/SC of the Nigerian Society. *Pharmacognosy.* 2003; 1-7.
- Bajaj, Y. P. S. Medicinal and aromatic plants, Biotechnology in agriculture and forestry. Berlin: Springer-Verlag. 1988; 24.
- Chawla AS, Singh M, Murthy MS, Gupta M, Singh H. Anti-inflammatory action of ferulic acid and its esters in carrageenan induced rat paw oedema model. *Indian J Exp Biol.* 1987; 25:187–189.
- Chhabra SC, Uiso FC, Mshiu EN. Phytochemical screening of Tanzanian medicinal plants. *J Ethnopharmacol.* 1984; 11:157–179.
- Choi HJ, Kim JH, Lee CH, Ahn YJ, Song JH, Baek SH, Kwon DH. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. *Antiviral Res.* 2009; 81:77–81.
- Das AK, Bhattacharjee A. A systematic approach to phytochemical screening. *Trop Sci.* 1970; 12: 54– 58.
- Delgado-Vargas F, Jimenez AR, Paredes-Lopez O. Natural Pigments: Carotenoids, Anthocyanins, and Betalains Characteristics, Biosynthesis, Processing, and Stability. *Critical Rev Food Sci Nutri.* 2000; 40:173–289.
- Evans D. Trease and Evans Pharmacognosy, 15<sup>th</sup> ed. WB Saunders company, Edinburgh. 2002; 334-337.
- Gibson EL, Wardel J, Watts CJ. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. *Appetite.* 1998; 31: 205-228.

Hahn NI. Is Phytoestrogens Nature's Cure for What Ails Us? A Look at the Research. *J Ame Diet Asso.* 1998; 98: 974-976.

Hamburger M, Hostettmann K. Bioactivity in Plants: The Link between Phytochemistry and Medicine. *Phytochemistry.* 1991; 30: 3864-3874.

Hammerschmidt R. PHYTOALEXINS: What Have We Learned After 60 Years? *Annu Rev Phytopathol.* 1999; 37: 285-306.

Harvey A. Strategy for discovering drugs from previously unexploited natural products. *Drug Discovery Today.* 2000; 5: 294-300.

Ismaili H, Sosa S, Brkic D, Fkih- Tetouani S, Ildrissi A, Touati D, Aquino RP, Tubaro A. Topical anti-inflammatory activity of extracts and compounds from *Thymus broussonettii*. *J Pharm Pharmacol.* 2002; 54: 1137- 1140.

Jorgensen KM. Pain assessment and management in the newborn infant. *J Perianesth Nurs.* 1999; 14: 349-356.

Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Adv Nutr.* 2011; 2(1): 32-50.

Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. *Phytochemistry.* 2005; 66 (17): 2056-2071.

Lu L, Liu SW, Jiang SB, Wu SG. Tannin inhibits HIV-1 entry by targeting gp41. *Acta Pharmacol. Sin.* 2004; 25 (2): 213-218.

Madarnas P, Benrezzak O, Pageau R, Nigam VN, Elhilali M. Evaluation of cortisone-heparin and cortisone-maltose tetrapalmitate therapies against rodent tumors. II. Pathological studies. *Anticancer Res.* 1989; 9: 1889-1895.

Mamta S, Jyoti S, Rajeev N, Dharmendra S and Abhishek G. Phytochemistry of Medicinal Plants. *J Pharmacogn Phytochem.* 2013; 1(6) 168-182.

Meagher E, Thomson C. Vitamin and Mineral Therapy in Medical Nutrition and Disease. 2nd ed. Blackwell Science Inc. 1999; 33-58.

Patocka J. Biologically active pentacyclic triterpenes and their current medicine signification. *J Appl Biomed.* 2003; 9: 7-12.

Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants *in vivo*. *Free Radic Res.* 1997; 26: 381-398.

Robert AM. Encyclopedia of Physical Science and Technology. 3<sup>rd</sup> ed. Academic press. 1992.

Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. *Biochem Pharmacol.* 2012; 83: 6-15.

Shinoda J. A new biologically active flavone glycoside from the roots of *Cassia fistula* Linn. *J Pharm Soc Jp.* 1928; 48: 214-220.

Soine TO. Naturally occurring coumarins and related physiological activities. *J Pharm Sci.* 1964; 53: 231-264.

Tan AC, Konczak I, Sze DM, Ramzan I. Towards the discovery of novel phytochemicals for disease prevention from native Australian plants: an ethnobotanical approach. *Asia Pac J Clin Nutr.* 2010; 19: 330-334.

Wang ZN, Wang MY, Mei WL, Han Z, Dai HF. A new cytotoxic pregnanone from *Calotropis gigantea*. *Molecules.* 2008; 12(12): 3033-3039.

Zhu L, Takahashi Y, Amagai T, Matsushita H. Highly sensitive automatic analysis of polycyclic aromatic hydrocarbons in indoor and outdoor air. *Talanta.* 1997; 45: 113-118.