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NIBMA PATRA (*AzardirachtaindicaA.Juss)* ALCOHOLIC EXTRACTION AND PHYTOCHEMICAL ANALYSIS- A REVIEW

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ABSTRACT

Herbal remedies are known to treat many infectious diseases throughout the history of mankind.*NimbaPatra*(*AzardirachtaindicaA.Juss*) is described as *Krimighna* in *Ayurveda* and researches show its antimicrobial effects against microorganisms. Therefore, Present study was planned to isolate active ingredients of *NimbaPatra*by alcoholic extraction with Soxhlet apparatus. Extraction is separation process for isolation of active components of any herbal plans or materials.25 gm of shade dried coarse powder of *NimbaPatra* and 250 ml of Ethyl alcohol as extraction solvent was taken. On fifth day the extraction process was completed. After evaporation of the alcoholic extract by water bath 2.00 gm of the alcoholic extract was obtained. The alcoholic extract of *NimbaPatra* was subjected to Phytochemical analysis to assess phytochemical constituents. Results ofphytochemical analysis showed presence of alkaloids, glycosides, saponins and sugar.

Keywords: Alcoholic extract, *NimbaPatra*, Soxhlet apparatus, Phytochemical Analysis.

INTRODUCTION

Plant material (Herbal remedies) continues to play a major role in the primary health care as therapeutic remedies. Thus, the discovery of medicinal plants as antimicrobial agents is useful in replacing wide varieties of antibiotics and evidence based better utilization of Ayurveda remedies.

WHO estimated that plant extracts or their active constituents are used as folk medicine in traditional therapies in 80% of the world's population.[1]About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful in the areas of infectious disease and cancer.[2]

Extraction refers to separation processes for the isolation of the active ingredients and components from drug material. The separation of useful components of herbal drugs and tissues using particular solvents through standard procedure are defined as extraction. Phytochemical screening refers to the extraction, screening and identification of the medically active substances found in plants.

In Ayurveda various drugs are mentioned with ascribed *Krimighna* action. *Nimba* is mentioned as *Krimighna* and *Krimihara* in Samhita by various

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Acharyas. Nimba is described to be useful against ulcers, wounds, skin diseases etc in folklore practice. *Nimba* leaf paste is applied to boils, ulcer, abscess, inflammation and other similar ailing conditions.[3]*Nimba* was selected because of its antimicrobial properties and ascribed actions. In Charaka Samhita *Nimba* is mentioned in *Kandugna* and *Tikta Skanda*[4]. It pacifies *Kapha* and *Pitta Dosha*,[5] prevents formation and growth of *Krimis*. *Nimba* (*AzardiractaindicaA.juss)* has antibacterial, antifungal, antiparasitic and antiviral propertie.[6]

In the present study alcoholic extraction of *NimbaPatra* by Soxhlet apparatus is dealt followed by phytochemical analysis. Thereby achieve extraction of alcoholic extract of *Nimba Patra* that can be further used in research like culture and sensitivity against microorganisms and also phytochemical analysis will be useful for better understanding of its therapeutic action.

MATERIAL AND METHODS

SOXHLET APPARATUS[7]

A Soxhlet extractor has three main sections: Percolator which circulates the solvent, Thimble that retains the solid to be laved and Siphon mechanism which periodically empties the thimble.

ASSEMBLY

1. The source material containing the compound to be extracted is placed inside the thimble.
2. The thimble is loaded into the main chamber of the Soxhlet extractor.
3. The extraction solvent to be used is placed in a distillation flask.
4. The flask is placed on the heating element.
5. The Soxhlet extractor is placed atop the flask.
6. A reflux condenser is placed atop the extractor.

PRINCIPLE OF SOXHLET APPARATUS

The solvent is heated to reflux. The solvent vapor travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser cools the solvent vapour and drips back down into the chamber, consisting of the solid material. The chamber containing the solid material slowly fills with warm solvent. In this chamber some of the desired compound gets dissolved in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the distillation flask. This cycle may be repeated many times.

During each cycle, a portion of the non-volatile compound gets dissolved in the solvent. After many cycles the desired compound is concentrated in the distillation flask. After extraction the solvent is removed, typically by means of a rotary evaporator yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimblewhich may be discarded.

DRUG COLLECTION

The fresh leaves of *Nimba* (*AzadirachtaindicaA.juss*) were collected from SriDharmasthalaManjunatheshwara College of Ayurveda & Hospital botanical garden. The fresh leaves were washed under tap water and were shade dried.The completely shade dried leaves was grounded to course powder. It was stored in a clean and air tight container.

AUTHENTICATION OF THE DRUG

The drug *NimbaPatra* (*AzadirachtaindicaA.juss)* authentication was done by the Department of Dravyaguna, in Sri DharmasthalaManjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka.

METHODOLOGY

Preparation of Alcoholic extract of *NimbaPatra (AzadirachtaindicaA.Juss*)

Instrument: Soxhlet apparatus

Requirements: Shade dried*NimbaPatra*

Coarse powder- 25 gm

Ethanol - 250ml

NIMBA PATRA ALCOHOLIC EXTRACTION[8]

25 gm of shade dried coarse powder of *NimbaPatra (AzadirachtaindicaA.Juss*) was placed inside a thimble made from thick filter paper which was loaded into the main chamber of the Soxhlet extractor. 250 ml of Ethyl alcohol as extraction solvent was taken in a distillation flask and the Soxhlet extractor was placed on this flask. The Soxhlet was then equipped with a condenser.

The solvent was heated to reflux. The solvent vapor traveled up a distillation arm, and flooded into the chamber housing the thimble of sample. The condenser ensured that any solvent vapor cools and drips back down into the chamber housing the solid material.

The chamber containing the sample slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber got automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat for five times, for five days till a light green color liquid was seen through a siphon side arm. The obtained alcoholic extract of *NimbaPatra* was subjected to water bath and evaporated to dryness and stored in air tight bottles at 4^0C for further use.

PHYTOCHEMICAL ANALYSIS OF ALCOHOLIC EXTRACT OF NIMBA PATRA

NimbaPatra (*AzadirachtaindicaA.Juss*) alcoholic extract was subjected to phytochemical analysis. Presence of alkaloids (Drangendorff's test), tannins (Lead acetate solution test), glycosides (Bornstrager's test), saponins (Foam test), and sugar (Benedict's test) were evaluated.

1. Test for Alkaloids[9]: 2-3 ml of alcoholic extract of *NimbaPatra* was added with few drops of Drangendorff's reagent in test tube. Orange brown precipitate indicates presence of Alkaloids in alcoholic extract of *NimbaPatra*.

2. Test for Tannins[9]: 2-3 ml of alcoholic extract of *NimbaPatra* was added with few drops of lead acetate solution in test tube. White precipitate indicates presence of Tannins in the alcoholic extract of *NimbaPatra*.

3. Test for Glycosides[10]: 5 ml of alcoholic extract of *NimbaPatra* was added with 5 ml.5% FeCl3 and 5ml dilute HCL heated for 5 minutes in boiling water bath. Cooled and added with benzene solvent shaked well. Organic layer separates. Added equal volume dilutes ammonia. Ammoniacal layer, Pinkish red color was not observed in test tube. This indicates absence of Glycosides in the alcoholic extract of *NimbaPatra*.

4. Test for Saponins[10]: Alcoholic extract of *NimbaPatra* was shaked with distilled water. Persistent foam shows presence of Saponins in alcoholic extract of NimbaPatra.

5. Test for Sugars[11]: Mixed equal volume of Benedict's reagent and Alcoholic extract solution in test tube. Heated in boiling water bath for 5 min. Solution turns green color in test tube indicates presence of sugarinalcoholic extract of *Nimba Patra*.

OBSERVATION AND RESULTS

As per above procedure 25 gm of shade dried coarse powder of *NimbaPa-*

tra(AzadirachtaindicaA.Juss) and 250 ml of Ethyl alcohol as extraction solvent was taken in Soxhlet apparatus.

Table 1: Alcoholic extraction of *NimbaPatra*bySoxhlet apparatus

Day	Date	Content	Time	Temperature
1	21/7/2015	Ethanol -250 ml *NimbaPatra*- 25 gm(coarse powder)	12:30 pm to 5:00 pm	60^{o}C
2	22/07/2015		9:00 am to 5:00 pm	55^{o}C
3	23/07/2015		8:30 am to 5:25 pm	60^{o}C
4	24/07/2015		9:00 am to 4:45 pm	60^{o}C
5	25/07/2015	Alcoholic Extractobtained– 230 ml Subjected to evaporation on water bath	9:45am to 5:30 pm	60^{o}C

Table2: After evaporation weight of alcoholic extract of *NimbaPatra*

Alcoholic Extract of *NimbaPatra*	Weight
Weight of empty vial	15.75 gm
Weight along with extract	17.75 gm
Extract after evaporation	2.00 gm

On the fifth day the extraction process was completed. 2.00 gm of the alcoholic extract was obtained after evaporation of the alcoholic extract by hotwater-bath.Obtainedalcoholic extract was solid in nature.

Table 3: Phytochemical Analysis of Alcoholic Extract of *NimbaPatra*

Sr.no	Phytochemical	Test name	Result
1	Alkaloids	Drangendorff's test	Present
2	Tannins	Lead acetate solution test	Present
3	Glycosides	Bornstrager's test	Absent
4	Saponins	Foam test	Present
5	Sugar	Benedict's test	Present

DISSCUSSION AND CONCLUSION

Soxhlet method is very simple and cheap. The advantages of conventional Soxhlet extraction include the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix, maintaining a relatively high extraction temperature with heat from the distillation flask and no filtration requirement after leaching. Alcohol provides a particularly effective way of maximizing the bioavailability of the actives extracted from the plant. Ethanol is a molecule with both the polar and non-polar ends. Ethanol can be used to extract both groups of compounds. In the present study Soxhlet extraction of 25 gram shade dried *Nimba Patra* along with 250ml of ethanol after evaporation yielded 2 gram of solid *Nimba Patra* extract which can further be used for research like culture and sensitivity to micro-organism etc. Phytochemical analysis

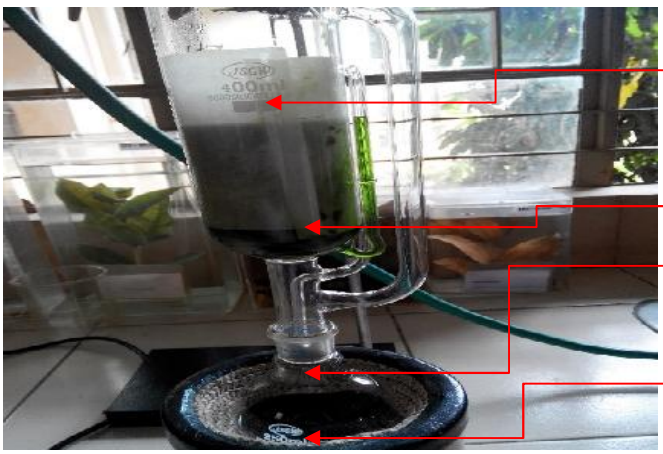
helps us to identify active principles in drug usually responsible for therapeutic action.

The phyto-constituents like alkaloids, glycosides,saponinsetc are active principles of the plant, responsible for therapeutic action. Saponin extracts are inhibitory to the growth of the Gram-positive bacteria[12] and alkaloids displays good antimicrobial activity against several test microorganisms.[13]The phytochemical analysis showed the presence of tannins, alkaloids, sugars and saponin in alcoholic extract of the *Nimba Patra* in present study.

NIMBA PATRA (WET)

NIMBA PATRA (DRY)

NIMBA PATRA
(COARSE POWDER)



Soxhletapperatus

***NimbaPatra* alcoholic extract before evaporation**

***NimbaPatra* alcoholic extract during evaporation**

***NimbaPatra* alcoholic extract after evaporation**

***NimbaPatra*alcoholic extract after evaporation**

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