Preparation and Evaluation of tincture of Azadirachta indica A. Juss. Leaves

SUBHASH C. MANDAL, SABITA PAL (DUTTA), TAPAN K. MAITY, M. PAL AND B.P. SAHA
Department of Pharmaceutical technology, faculty of engineering and Technology Jadavpur university Calcutta - 700032

Received: 12. 5. 1998                                                                                   Accepted: 30.12.1998

ABSTRACT : Different strength of alcohol 40.50,60,70,80,90 per cent (v/v) and absolute were utilized for the preparation of tincture of azadirachta Indica A. Juss leaves by maceration for 7, 14,21 days. 60 per cent tincture produced after 21 says of maceration showed a maximum solid content of 1.425 per cent (v/v) and sowed pH 3.15. This tincture was characterized by studying is various physico-chmicla properties along wit its thin-layer chromatographic characteristics.

INTRODUCTION

Azadirachta indica A. Juss. Syn Melia azadirachta Linn. (Fam. Meliaceae) is a genus of tall, evergreen trees, native to India, is now widely distributed throughout the indo-Malayan region and is also found in tropical africa1.

Almost every part of this plant is used in Indian traditional system of medicine. The bark is bitter, astringent, acrid, refrigerant, depurative, antiperiodic, vulnerary, demulcent, liver tonic, expectorant, urinary astringent, anthelmintic, pectoral tonic and insecticidal2.

The leaves are bitter, astringent, acrid, depurative, antiseptic, anthelmintic, alextetric, appetizer, insecticidal, demulcent and refrigerant. They are useful in vitiated conditions of pitta, burning sensation, leprosy, skin diseases, leucoderma, pruritus, ophthalmopathy, intestinal worms, dyspepsia, ulcers, tuberculosis, boils, eczema and malarial and intermittent fevers2.

The flowers are bitter refrigerant, stomachic, anthelmintic and tonic.

The seeds are bitter, acrid, thermogenic, purgative, emollient, anodyne, anthelmintic, depurative, vulnerary, uterine stimulant, and urinany astringent.

Characterization of tinctures of medicinal plants like Ficus racemosa Linn3, Asparagus racemosus willd4, Andrographis peniculata nees5, Embelia ribes burma6 and Lucas lavandulaefolia Rees7 has already been reported.

In the present investigation we have tried to find our the suitable strength of alcohol to prepare the tincture of A. indica leaves and evaluate the same by studying the possible chang4es in pH during maceration process, maximum yield of total solids, number of days required for effective maceration, chemical group tests and thin-layer chromatographic characterization of the
tinctures which yielded maximum total solid.

MATERIALS AND METHODS

Plant material

The leaves of *A. indica* were collected locally and it was identified by pharmacognosy division of our department of Jadavpur University. The leaves were dried, powdered and passed through 40 mesh sieve and kept for further uses.

CHEMICALS

All materials used were of analytical reagent grade. Absolute alcohol was obtained from Bengal chemical and pharmaceutical Ltd. Calcutta and from that different dilutions were made with distilled water.

METHODS

Twentyone amber coloured bottles were taken, 200 ml of alcohol of following strengths; 40, 50, 60, 70, 80, 90 per cent (v/v) and absolute were poured in separate three sets of seven bottles, each containing specific amount of powdered leaves. Each set of seven bottles were kept for maceration for 7, 14 and 21 days respectively. The materials were filtered through sintered glass crucible (G-4) after maceration.

The physical constant values such as colour, pH, specific gravity, solid matter per 100ml were studied, the chemical group tests 8, 9 tin layer chromatographic characteristics were studies 10.

RESULTS AND DISCUSSION

The colours of tinctures prepare were same in the tinctures prepared by 7, 14 and 21 days of maceration. All the tinctures were clear liquid without any turbidity or sedimentation. Three sets of tinctures were prepared by maceration process utilizing multiples of 10 per cent strengths of dilute alcohol. The products were kept for 7, 14 and 21 days and total solid content for the respective days were studied and noted accordingly.

The colour of tinctures were varied with different strengths of alcohol. This may be due to the variations of the metabolites present in the leaves. The colour was same in such set for a particular strength of alcohol (Table 1).

The variations of pH of the tinctures prepared at different days of maceration were quite distinct (table 2). Such change indicates the variations in total solid content and chemical constituents present in them. This change may occur due to prolonged contact with the glass container.

Specific gravity of different tinctures with duration of maceration are shown in Table 3. It is observed that specific gravity is maximum (0.978) with 40 per cent alcohol and minimum (0.831) with absolute alcohol after 21 days of maceration.

The total solid content is maximum (1.425) at 60 per cent alcohol on 21 days of maceration and it is decreased with increase of alcohol strength (table 4).

In chemical group tests of the tincture with 60 per cent (v/v) alcohol the presence of alkaloid, sterol, tannin, saponin, flavonoid and reducing suger are confirmed.

In thin-layer chromatographic study it is observed at the tincture prepared with 60 per cent alcohol gave five spots in chloroform: acetone (9:1) solvent system and five spots in chloroform: absolute ethanol: 25%
ammonia solution (9:1:1) solvent system (Tables 6,7).

We are thankful to the authority of Jadavpur University for providing all facilities

ACKNOWLEDGEMENT

REFERENCE:

1. Anonymous, The Wealth of India, Raw Materials, Vol. 1. Publication and Information directorate, C.S.I.R., New Delhi. Vol. I, 504- 511 (1895)

2. Warrier, P.K. Nambiar V.P.K., and Ramankutty, C., Indian medicinal plants, Vol 1, orient Longman, madras, 203 – 208 (1993).

3. Mandal S.C., Mukherjee P.K, Pal M., and saha B.P., Anc Sci Life XVI, 3, 196-199 (1997).

4. Mandal S.C., Mukherjee P.K, Nandy A., Pal M., and saha B.P., Anc Sci Life XVI, 2, 160-165 (1996).

5. Pal S.K. Chattaraj S.C., and saha B.P., Res & Ind., 38, 154 (1993).

6. Pal S.K., Mukherjee P.K., and saha B.P., Res & Ind, 39, 253 (1994).

7. Mukherjee P.K., Saha K., Perumol P., Pal S.K., Pal M., and saha B.P., Sci & Ind. Res., 55, 286 (1996).

8. Tyler, V.E., Brady L.R., Robbers J.E., Pharmacognosy 9th Edn. Lea & Febiger publication, Philadelphia (1985).

9. Trease G.E. and Evans W.C., Pharmacognosy, 12th Edn., ELBS publication (1985).

10. Stahl E., Tin-layer chromatography, 2nd Edn., Springer – Verlag, New York (1969).

**Table 1- Colour description of the tinctures**

| Per cent of alcohol (v/v) | Colour of the prepared tinctures |
|--------------------------|----------------------------------|
| 40                       | Light brown                      |
| 50                       | Brown                            |
| 60                       | Deep brown                       |
| 70                       | Gryish brown                     |
| 80                       | Greenish brown                   |
| 90                       | Greenish brown                   |
| Absolute                 | Greenish brown                   |
Table -2 pH of the tinctures

| Alcohol strength (% v/v) | pH (with duration of maceration) |
|--------------------------|----------------------------------|
|                          | 07 days | 14 days | 21 days |
| 40                       | 3.20    | 3.10    | 3.10    |
| 50                       | 3.25    | 3.10    | 3.10    |
| 60                       | 3.25    | 3.15    | 3.15    |
| 70                       | 3.50    | 3.25    | 3.30    |
| 80                       | 3.40    | 3.35    | 3.30    |
| 90                       | 3.50    | 3.35    | 3.25    |
| Absolute                 | 3.45    | 3.40    | 3.30    |

(Each reading is an average of three determinations)

Table 3- Specific gravity of the tincture

| Alcohol strength (% v/v) | Sp. Gravity (with duration of maceration) |
|--------------------------|------------------------------------------|
|                          | 07 days | 14 days | 21 days |
| 40                       | 0.977   | 0.977   | 0.978   |
| 50                       | 0.969   | 0.970   | 0.971   |
| 60                       | 0.960   | 0.958   | 0.961   |
| 70                       | 0.930   | 0.929   | 0.932   |
| 80                       | 0.919   | 0.917   | 0.916   |
| 90                       | 0.885   | 0.888   | 0.886   |
| Absolute                 | 0.832   | 0.832   | 0.831   |

(Each reading is an average of three determinations)

Table 4- Solid matter content of the tincture

| Alcohol strength (% v/v) | Solid matter content (% w/v) (with duration of maceration) |
|--------------------------|------------------------------------------------------------|
|                          | 07 days | 14 days | 21 days |
| 40                       | 0.995   | 1.050   | 1.080   |
| 50                       | 1.015   | 1.070   | 1.125   |
| 60                       | 1.190   | 1.300   | 1.425   |
| 70                       | 1.040   | 1.210   | 1.300   |
| 80                       | 1.045   | 1.235   | 1.325   |
| 90                       | 1.000   | 1.075   | 1.102   |
| Absolute                 | 0.917   | 1.025   | 1.050   |

(Each reading is an average of three determinations)
Table 5 – Chemical group tests on 60% (v/v) tincture

| Chemical group   | Result |
|------------------|--------|
| Alkaloid         | +      |
| Flavonoid        | +      |
| Reducing sugar   | +      |
| Sterol           | +      |
| Saponin          | +      |
| Tannin           | +      |
| Anthraquinone    | -      |

‘+’ present ‘-’ Absent

Table 6- Thin-layer chromatographic characteristics of the tincture
Prepared with 60% (v/v) alcohol

(i) Solvent system : Chloroform: acetone (9:1)  
(ii) Stationary phase : Silica gel G.  
(iii) Room temperature : 30°C  
(iv) Spraying reagent : dragendorff’s reagent

| No. of spot | Colour before spraying | Colour after spraying and heat | R_f value |
|-------------|-------------------------|--------------------------------|-----------|
|             | Day light | UV light                        |           |
| 1           | Pink      |                                | 0.26      |
| 2           | Light blue|                                | 0.52      |
| 3           | Yellowish | Light pink                      | 0.56      |
| 4           | Yellow    | Deep pink                       | Brownish Orange | 0.67 |
| 5           | Greenish blue | Blue                           | 0.97      |

Table 7- Thin-layer chromatographic characteristics of the tincture
Prepared with 60% (v/v) alcohol

(i) Solvent system : Chloroform : absolosolute ethanol : 25% ammonia (9:1:1)  
(ii) Stationary phase : Silica gel G.  
(iii) Room temperature : 30°C  
(iv) Spraying reagent : dragendorff’s reagent

| No. of spot | Colour before spraying | Colour after spraying and heat | R_f value |
|-------------|-------------------------|--------------------------------|-----------|
|             | Day light | UV light                        |           |
| 1           | Light blue |                                | 0.42      |
| 2           | Blue      |                                | 0.53      |
| 3           | Light pink|                                | 0.68      |
| 4           | Yellow    | Light brown                     | Brownish Orange | 0.75 |
| 5           | Green     | Green                           | 0.88      |