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An Assessment of Variation in Active Ingredients of Ampucare from Different Zones of India

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ABSTRACT

The present study was designed to assess the variation in curcumin content of *Curcuma longa* rhizome and total polyphenols in *Azadirachta indica* bark samples procured from different zones of India. Physico-chemical tests such as total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, volatile oil content etc. were also determined. A slight variation was seen in the bark samples of *Azadirachta indica.* Total ash ranged from 4.25±0.15 (East zone) to 5.57±0.20 (Central zone) whereas acid insoluble ash ranged from 0.80±0.07 (East zone) to 1.52±0.06 (Central zone). Alcohol soluble extractive value of East zone sample was found to be more than 4 times higher 16.95±0.80 than that of central zone 3.85±0.12, where lowest value was recorded. Water soluble extractive value was also found to be highest in East zone sample 17.80±1.10 which was 2 times more than that of Central zone sample 8.45±0.15. This variation may be due to variation in climatic conditions, soil type, pollution stress etc. All the results were within the limits as given in The Ayurvedic Pharmacopoeia of India. Total Polyphenols ranged from 190.0 mgGAE/g of dry weight (Central zone) to 510.0 mgGAE/g of dry weight (East zone). All the samples were found to be rich in total polyphenols. In *Curcuma longa* samples, Total ash ranged from 3.10±0.20 (South zone) to 4.80±0.25 (North zone) whereas acid insoluble ash ranged from 0.55±0.04 (South zone) to 1.24±0.06 (North zone). Alcohol soluble extractive value was found to be highest in South zone sample 14.80±0.30 which was found to be more than 3 times higher than that of central zone 4.28±0.19 where lowest value was recorded. Water soluble extractive value was also found to be highest in West zone sample 12.55±0.69 and lowest in Central zone sample 8.90±0.37. Volatile oil ranged from 3.50±0.21 North zone sample to 5.50±0.20 South zone sample, where highest volatile oil was recovered. All the results were within the limits as given in The Ayurvedic Pharmacopoeia of India. In our study, curcumin content ranged from 0.30 % (Central zone) to 3.24 % (South zone). Average curcumin content was found to be 1.54 % on dry weight basis. Ampucare is prepared by selection of best quality herbs.

Keywords: Ampucare, *Curcuma longa*, *Azadirchta indica*, Total ash, Curcumin, Total polyphenols.

INTRODUCTION

Ampucare is a result oriented polyherbal formulation which has been proved to be a panacea for chronic non-healing wounds including diabetic leg ulcer, bed sores & burns. The wonderful properties of Ampucare are mainly due to the presence of *Azadirachta indica* and *Curcuma longa* as active ingredients.

Curcuma longa Linn. (Family: Zingiberaceae) has been reported to be antifungal, antibacterial, antiseptic and anti-inflammatory. [1-4] Due to these properties, it is used as a therapeutic agent in wound healing. [5] The therapeutic value of this herb is mainly due to the presence of curcumin which is a phenolic compound and is responsible for the yellow colour of turmeric. [6] It acts by modulating the activation of various transcription factors, regulates the expression of inflammatory enzymes, cytokines, adhesion molecules and cell survival proteins. [5] It improves wound healing by modulating collagen, decreasing reactive oxygen species or by increasing fibroblast and vascular density in wounds. [7-8]

Azadirachta indica (Family: Meliaceae) is a multipurpose tree in India. Bark of *Azadirachta indica* is being used by several Indian tribes as an antifungal, antiseptic, astringent in several skin diseases, boils and blisters, eczema etc. from centuries. [9] Modern scientific research has proved it to be anti-microbial, fungistatic, fungicidal, anti-inflammatory, antioxidant, acaricidal and free radical scavenger; useful in ulcers, infections and skin diseases. [10-14] Bark of *Azadirachta indica* contains polyphenols which are antioxidant phytochemicals that tend to prevent or neutralize

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the damaging effects of free radicals. [15] They are well known powerful antioxidants that scavenge free radicals, promote dermal fibrosis, act as an anti-oxidant, and improve wound healing and acts as an anti-inflammatory reagent. [16-18]

This study was undertaken to assess the curcumin content in *Curcuma longa* and total polyphenols in *Azadirachta indica* bark samples procured from different zones of India, along with some physico-chemical properties also.

MATERIALS AND METHODS

Procurement of samples

Curcuma longa and *Azadirachta indica* samples were procured from north, south, east, west and central zones of India (Table 1).

Reagents

All the solvents, reagents and standards (Curcumin and gallic acid) were purchased from Sigma-Aldrich, India. Folin-Ciocalteu's phenol reagent was purchased from E Merck, India.

Physico-chemical tests

All the physico-chemical tests were performed according to the Ayurvedic Pharmacopoeia of India. [19]

Description: Description of the plant part is given by observing the plant part with a naked eye or with the aid of a magnifying lens. It includes size, shape, outer and inner surface as well as sensory or organoleptic characters such as colour, odor and taste.

Foreign matter: Weigh 100-500 g of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and calculate the percentage present.

Moisture content: It is calculated by the following:

$$\text{Moisture content (\%)} = \frac{\text{Factor} \times \text{vol. of KF used} \times 100}{\text{Weight of sample (in g)} \times 1000}$$

Where KF = Karl Fischer reagent

Total ash: Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450°C degree until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°C. Calculate the percentage of ash with reference to the air-dried drug.

Acid insoluble ash: Boil the total ash for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Alcohol soluble extractive: Take 2 g of the coarsely powdered air dried drug in a conical flask. Add 50 ml of alcohol and extract it in a water bath for 15 minutes at 70°C. Repeat this process two more times until the colour of the extract becomes colourless. Filter rapidly, taking precautions against loss of solvent, evaporate the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

***Water soluble extractive*:** Take 2 g of the coarsely powdered air dried drug in a conical flask. Add 50 ml of distilled water and extract it in a water bath for 15 minutes at 70°C. Repeat this process two more times until the colour of the extract becomes colourless. Filter rapidly, taking precautions against loss of solvent, evaporate the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105 degrees, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Volatile oil: The determination of volatile oil in a drug is made by distilling the drug with a mixture of water and glycerine, collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w. The drug in the form of powder, is placed together with 75 ml of glycerin and 175 ml of water in the one liter distilling flask, and a few pieces of porous earthern ware and one filter paper 15 cm cut into small stripes, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into the graduated receiver, keeping the tap open until the water overflows, at P. Any air bubbles in the rubber tubing are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap is opened and the tube lowered slowly; as soon as the layer of the oils completely enters into the graduated part of the receiver the tap is closed and the volume is read.

The tube is then raised till the level of water in it is above the level of B, when the tap is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ. The measured yield of volatile oil is taken to be the content of volatile oil in the drug.

Estimation of Curcumin by spectrophotometric method

It was done according to the method prescribed by IS: 10925-1984. [20] Weigh accurately 0.1 g turmeric extract or 0.005 g to 0.01 g curcuminoids in 25 ml ethyl alcohol. Warm it if necessary for complete dissolution, filter through Whatman No. 41 paper, wash the filter paper with little alcohol and transfer it to 100 ml volumetric flask. Make up the volume with ethyl alcohol and pipette 10 ml to another 100 ml volumetric flask, make up the volume with alcohol. Measure the absorbance at 425 nm in 1 cm cell against an alcohol blank.

Calculations: A standard solution of curcumin 0.0025 g/l gives absorbance value of 0.42 at 425 nm.

$$\text{Absorptivity of curcumin (A)} = \frac{0.42}{1 \times 0.0025}$$

$$\text{\% of curcumin in extract} = \frac{a \times 100}{l \times A \times W}$$

Where, a = absorbance at 425 nm; l = cell length in cm; A = absorptivity; W = Wt of sample in g

Estimation of total polyphenols

The total phenolic content of the herbal extracts was estimated according to the method given by Singleton and Rossi. [21] Ten milligrams of standard Gallic acid (purchased from Sigma (Aldrich) India Pvt. Ltd.) was dissolved in 100 ml distilled water in a volumetric flask (100g/ml of stock solution). From this stock solution, 0.5 to 2.5 ml of aliquots was pipetted out into 25 ml volumetric flasks. Ten ml of distilled water and 1.5 ml of Folin Ciocalteu's reagent were added to each of the above volumetric flasks. Four ml of 20 % sodium carbonate solution was added after 5 minutes and the volume was made up to 25 ml with distilled water and incubated at room temperature for 30 minutes and the absorbance of the solution was recorded at 765 nm and a standard curve of absorbance verses concentration of Gallic acid was plotted.

One gram of the powdered material was extracted with 95 % ethanol (thrice), filtered with a 1.2 micron filter paper and was adjusted to 50 ml with 95 % ethanol in a volumetric flask. From this stock solution the same steps were repeated as given below for the preparation of standard. Percentage of total phenolics was calculated from calibration curve of Gallic acid and total phenolics were expressed as mg Gallic acid equivalents/gram (mgGAE/g) of dry weight.

RESULTS AND DISCUSSION

Ampucare contains *Curcuma longa* Linn. and *Azadirachta indica* Juss. as major ingredients. Bark samples of *Azadirachta indica* procured from different zones of India, were subjected to various physico-chemical tests (Table 2). External surface of all the barks were almost rough, fissured and rusty-grey whereas slight variation in the inner surface was seen in some samples (Table 2). Fracture was fibrous and odor was characteristic in all the samples analyzed. Taste was bitter and foreign matter was nil (Table 2). Moisture content ranged from 3.20±0.19 (East zone) to 5.25±0.25 (Central zone). Total ash ranged from 4.25±0.15 (East zone) to 5.57±0.20 (Central zone) whereas acid insoluble ash ranged from 0.80±0.07 (East zone) to 1.52±0.06 (Central zone). Extractive values give a sum of active constituents present in the crude herb. Alcohol soluble extractive value of East zone sample was found to be more than 4 times higher 16.95±0.80 than that of central zone 3.85±0.12, where lowest value was recorded. Water soluble extractive value was also found to be highest in East zone sample 17.80±1.10 which was 2 times more than that of Central zone sample 8.45±0.15. This variation may be due to variation in climatic conditions, soil type, pollution stress etc. All the results were within the limits as given in The Ayurvedic Pharmacopoeia of India. [22]

Bark of *Azadirachta indica* were analyzed for their polyphenol content also. Plant polyphenols are secondary metabolites in plant, containing many phenol-hydroxyl groups. They can bind with protein, alkaloids and polysaccharides, and consequently, thus exhibit an astringent property. [23] Polyphenol rich extract heals the wound much faster by the improved rate of wound contraction and decreased time taken for epithelialization. [24] They are responsible for modulation of angiogenesis also [25] and possess the property of stabilization of collagen also. [26]

Total Polyphenols ranged from 190 mgGAE/g of dry weight (Central zone) to 510 mgGAE/g of dry weight (East zone). All the samples were found to be rich in total polyphenols.

Rhizomes of *Curcuma longa* procured from different zones of India, were subjected to various physico-chemical tests (Table 1&3). External surface of all the rhizomes were round to oval or cylindrical with root scars; yellow to deep yellow or orange-red (Table 3). Fracture was horny; odor and taste was characteristic in all the samples studied (Table 3).

Moisture content ranged from 2.05±0.10 (South zone) to 2.50±0.18 (Central zone). Total ash ranged from 3.10±0.20 (South zone) to 4.80±0.25 (North zone) whereas acid insoluble ash ranged from 0.55±0.04 (South zone) to 1.24±0.06 (North zone).

Alcohol soluble extractive value was found to be highest in South zone sample 14.80±0.30 which was found to be more than 3 times higher than that of central zone 4.28±0.19 where lowest value was recorded. Water soluble extractive value was also found to be highest in West zone sample 12.55±0.69 and lowest in Central zone sample 8.90±0.37. Volatile oil ranged from 3.50±0.21 North zone sample to 5.50±0.20 South zone sample, where highest volatile oil was recovered. All the results were within the limits as given in The Ayurvedic Pharmacopoeia of India. [22]

Different samples of *Curcuma longa* were subjected to spectrometric estimation also. According to one study, curcumin content of pure turmeric powder was found to have

Table 1: Procurement of samples from different zones of India

Name of Ingredient	North zone	South zone	East zone	West zone	Central zone
Azadirachta indica (Bark)	Lucknow (U.P.)	Chennai (Tamil Nadu)	Kolkata (West Bengal)	Mumbai (Maharashtra)	Bhopal (M.P.)
Curcuma longa (Rhizome)	Lucknow (U.P.)	Chennai (Tamil Nadu)	Kolkata (West Bengal)	Mumbai (Maharashtra)	Bhopal (M.P.)

Table 2: Variation in physico-chemical characteristics in *Azadirachta indica* procured from different sources

Test	North zone	South zone	East zone	West zone	Central zone
External surface	Rough, fissured and rusty-grey	Rough, fissured and rusty-grey	Rough, fissured and rusty-grey	Rough, fissured and rusty-grey	Rough, fissured and rusty-grey
Inner surface	Yellowish and foliaceous	Creamish-yellow and foliaceous	Pinkish-red and foliaceous	Light brown to yellow and foliaceous	Yellowish and foliaceous
Fracture	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Bitter	Bitter	Bitter	Bitter	Bitter
Foreign matter	Nil	Nil	Nil	Nil	Nil
Moisture content	3.50±0.22	3.80±0.15	3.20±0.19	4.90±0.17	5.25±0.25
Total ash	5.10±0.24	4.98±0.16	4.25±0.15	5.52±0.19	5.57±0.20
Acid insoluble ash	1.10±0.03	1.00±0.03	0.80±0.07	1.17±0.05	1.52±0.06
Alcohol soluble extractive	13.25±0.40	12.45±0.14	16.95±0.80	8.85±0.17	3.85±0.12
Water soluble extractive	13.44±0.19	11.01±0.58	17.80±1.10	10.50±0.17	8.45±0.15
Total polyphenols mgGAE/g	416 mg GAE/g	241 mg GAE/g	510 mg GAE/g	397 mg GAE/g	190 mg GAE/g

Values are mean ± S.D. of 3 values each

Table 3: Variation in physico-chemical characteristics in *Cucuma longa* procured from different zones

Test	North zone	South zone	East zone	West zone	Central zone
External surface	Round to oval with root scars and annulations of leaf bases; dull yellow	Round to oval with less root scars and annulations of leaf bases; deep yellow to orange-red	Round to cylindrical with root scars and annulations of leaf bases; yellowish to yellowish-brown	cylindrical with root scars and annulations of leaf bases; yellowish to yellowish-brown	Cylindrical with prominent root scars and annulations of leaf bases; yellowish
Fracture	Horny	Horny	Horny	Horny	Horny
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Foreign matter	Nil	Nil	Nil	Nil	Nil
Moisture content	2.30±0.31	2.05±0.10	2.35±0.17	2.23±0.12	2.50±0.18
Total ash	4.80±0.25	3.10±0.20	3.97±0.25	3.60±0.17	4.75±0.20
Acid insoluble ash	1.24±0.06	0.55±0.04	0.80±0.09	0.60±0.04	0.67±0.08
Alcohol soluble extractive	6.75±0.18	14.80±0.30	10.03±0.25	8.95±0.18	4.28±0.19
Water soluble extractive	10.76±0.50	11.01±0.45	11.25±0.28	12.55±0.69	8.90±0.37
Volatile oil	3.50±0.21	5.50±0.20	4.80±0.23	4.50±0.30	3.80±0.15
Curcumin% (In powder)	1.16 %	3.24 %	1.67 %	1.33 %	0.30 %

Values are mean ± S.D. of 3 values each

average curcumin concentration of 3.14 %. [27] Variation in curcumin contents and oil quality in the land races of turmeric of north Indian plains has been reported where curcumin content varied from 0.61 % to 1.45 % on a dry weight basis. [28] Variation in curcumin content may be due to various factors such as effect of growth period, maturity of plant, storage time and varieties. [29] A decrease in pigment production as a function of maturity of the rhizomes of *Curcuma longa* was observed by Teonnesen et al. [30] Local climate, season, soil, water, pollution and stress are some other factors responsible for such variations. [31-32]

In our study, curcumin content ranged from 0.30 % (Central zone) to 3.24 % (South zone). Average curcumin content was found to be 1.54 % on dry weight basis.

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