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RESEARCH ARTICLE

PHARMACOGNOSY

QUALITY CONTROL ASPECTS OF THE WHOLE PLANT CALTROP

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ABSTRACT

Standardization of herbal is important to meet the WHO parameters. A comparison of methanolic extracts of fruits of *Tribulus terrestris* Linn roots, fruits and root powder were done with whole plant. Proximate analysis of fruit powder *Tribulus terrestris* Linn were compared with the reported values of proximate analysis. The whole plant powder extract in HPTLC method indicated that the band with Rf value of 0.65 was observed in each developed and scanned chromatographic plate. The other bands present in *Tribulus terrestris* Linn methanolic extract of fruits, roots and leaves were found to be different and hence it is possible to differentiate plant parts of *Tribulus terrestris* Linn. A study of percentage composition of various phyto chemical aspects such as total ash, acid insoluble ash, and nitrate content in *Tribulus terrestris* Linn was performed. Nitrate content in fruit powder showed linearity curve using Double Beam UV Spectrophotometry and Colorimetry.

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KEYWORDS

Tribulus terrestris Linn, Total ash, Acid insoluble ash, Nitrate content, HPTLC, Double beam UV Spectrophotometer .

INTRODUCTION[1,2]

Tribulus is also a derivative Roman era (Latin language) name for the weapon known in English today as the Caltrop, which bears strong resemblances with the plant today named Latin. *Tribulus terrestris* or puncture vine or gokhuru. Parts used are whole plant and seeds. The best-known member is *T. terrestris* (puncture vine), a widespread weed and also the source of a dietary supplement claimed to increase the body's natural testosterone levels and thereby improve male sexual performance and help to build muscle. *Tribulus* has been shown to enhance sexual behaviour in an animal model. It appears to do so by stimulating androgen receptors in the brain. Some species are cultivated as ornamental plants in warm regions. Some, notably *T. cistoides, T. longipetalus, T. micrococcus*, *T. terrestris, and T. zeyheri*, are considered weeds. *Tribulus terrestris* is a flowering plant in the family Zygophyllaceae, native to warm temperate and tropical regions of the Old World in southern Europe, southern Asia, throughout Africa, and in northern Australia. It can thrive even in desert climates and poor soil. Like many weedy species, this plant has many common names. Puncture vine, Caltrop, Yellow Vine, and Goat head are the most widely used. *Tribulus terrestris* has long been a constituent in tonics in Indian ayurveda practice, where it is known by its Sanskrit name, *"gokshura."* It's also used as an aphrodisiac in Ayurveda. *Tribulus terrestris* increases testosterone by increasing gonadotropin-releasing hormone with gonadotropic adaptogen compound contained in *Tribulus terrestris* (GnRH) which in turn stimulates the production of LH and follicle-stimulating hormone (FSH). Testosterone, besides its role in muscle-building and raising fertility and libido, is known to have a positive effect on bone marrow activity (for red blood cell production) and the immune system.

MATERIALS AND METHODS

Methanol(AR)–Glaxosmithkline, Mumbai, whatmann filter paper – whatman international ltd., England. Hot plate – Apollo surgical instruments, Chennai, Electric Bunsen – Toshinwal, Chennai, Double beam UV spectro photometry – ELICO, Hyderabad. HPTLC – CAMAG, switzerland, colorimeter, ELICO, Hyderabad.

PREPARATION OF TRIBULUS TERRESTRIS LINN IN PLANT EXTRACT[3,4]

FRUIT EXTRACT

10gm of gokhuru fruits were accurately weighed and dissolved in 50ml of methanol 99.5% then macerated for 15 min. After the solution is makeup to 100 ml with methanol 99.5%, then the solution is filtered using whattman filter paper. The filtrate obtained was then concentrated on hot plate and stored in desiccator, which is used for further analysis.

LEAVES EXTRACT

2.4gm of gokhuru leaves were accurately weighed and dissolved in 40ml of methanol 99.5%. Then macerated for 15min, after that the solution is made up to 80 ml with methanol. Then the solution was filtered using whattman filter paper. The filtrate obtained was concentrated on hot plate and stored in desiccator and used for further analysis.

ROOTS EXTRACT

3gm of Gokhuru roots were accurately weighed and dissolved in 40ml of methanol 99.5 %.

Then macerated for 15min, after that the solution is made up to 80 ml with methanol. Then filter the solution using whattman filter paper. The filtrate obtained was concentrated on hot plate and stored in desiccator and used for further analysis.

PROXIMATE ANALYSIS OF FRUIT POWDER[1,2]

The preliminary phytochemical investigations included proximate analysis of *Tribulus terrestris* Linn fruits. The tests carried out for the proximate analysis included determination of moisture content, total ash and acid insoluble ash.

PROCEDURE FOR MOISTURE CONTENT

Moisture content of drug can be employed a method of loss on drying (LOD) for the study of moisture level. The major loss in weight at the boiling temperature of water is although principally due to water, the volatile oil if present may also contribute to this weight loss. The moisture balance used for checking the LOD usually combine both a process of drying and its simultaneous weight recording up to the point of constant weight.

5gm of powdered drug is accurately weighed and spreaded on a china dish. Then it is heated on a water bath for 30min.Then the residue is weighed. The difference in weight will give the loss on drying that is the moisture content of the drug.

PROCEDURE FOR TOTAL ASH

Place about 2-4gm of ground air material, accurately weighed, in previously ignited and tared crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the temperature to 500-600 degree centigrade until it is white indicating the absence of carbon. Cool and weigh it. If carbon free ash can't be obtained in this manner cool the crucible and moistened the residue with about 2ml of water or saturated solution of ammonia nitrate. Dry on a water bath, then on a hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30min. then weigh without delay. Calculate the total ash in mg/gm of air dried material.

PROCEDURE FOR ACID INSOLUBLE ASH

Take 0.4gm of ash obtained earlier in a crucible and add 25ml of Hydrochloric acid (70gm/lit). Cover with watch glass and boiled gently for five min. rinse watch glass with 5ml of hot water and add this liquid to the crucible. Collect the insoluble mattes on the ash less filter paper and wash with hot water until filtrate is neutral, transfer the filter paper containing insoluble matter to the original crucible. Dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30min. then weigh with out delay. Calculate the content of acid insoluble ash in mg/gm of air dried material.

PROCEDURE FOR NITRATE CONTENT[1,7]

Accurately weighed 0.25gm of methanolic extract of drug was transferred to a 250ml standard volumetric flask and dissolved in distilled water and the volume was made up to the mark with distilled water and filtered. The filtered solution was transferred to five 100ml standard volumetric flasks as10ml, 20ml, 30 ml, 40ml, and 50ml and all five standard volumetric flasks were make up the volume up to the mark with distilled water. All five serial diluted standard volumetric flasks were kept in dark place for 24 hours.

A calibration curve was developed in the concentration range of 0ppm to 1ppm at wave length 570nm.

HPTLC - METHOD[1,9]

The instrument used in the present study was CAMAG-HPTLC system (Switzerland) comprising Camag linomat V automatic sample applicator, Camag TLC Scanner II with software. The samples were spotted in the form of bands of width 10mm using a camag

microlitre syringe on precoated silica gel 60 F 254 TLC precoated aluminum plates (E.Merck), 10cm width and 10cm length with 200 micrometer thickness using a Camag V applicator. A constant application rate of 5µl/s was employed and space between two bands was 5mm. The slit dimension was kept at 4mm length and 0.1 mm width and 20mm/s scanning speed was employed. The mobile phase consists of Ethyl acetate: acetone: Hexane (6:2:2), chromatogram was developed in a Camag twin trough glass chamber using a linear ascending technique. The chamber saturation time for mobile phase was optimized to 30min at room temperature. The length of chromatogram run was 80mm. densitometry scanning was performed on Camag TLC scanner II in the absorbance mode at 254nm. The source of radiation utilized was deuterium lamp.

RESULTS

Fig No:1

HPTLC Chromatogram of Tribulus terrestris leaf

Fig No : 2

HPTLC Chromatogram of Tribulus terrestris fruit

Fig No : 3

HPTLC Chromatogram of Tribulus terrestris root

Fig:No:4

Integrated chromatogram of different parts of Tribulus terrestris

Fig:No:5

Linearity curve of Tribulus terrestris by colorimetry

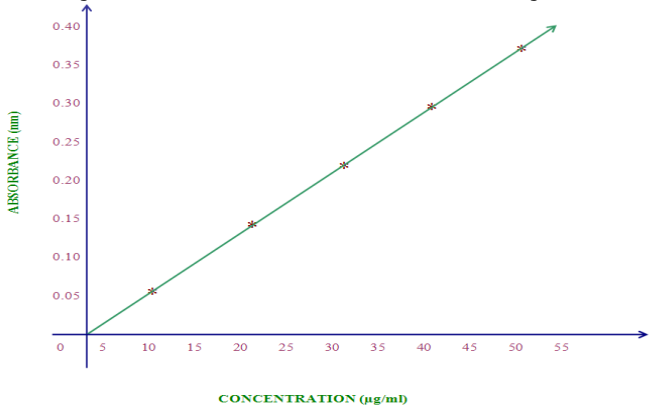
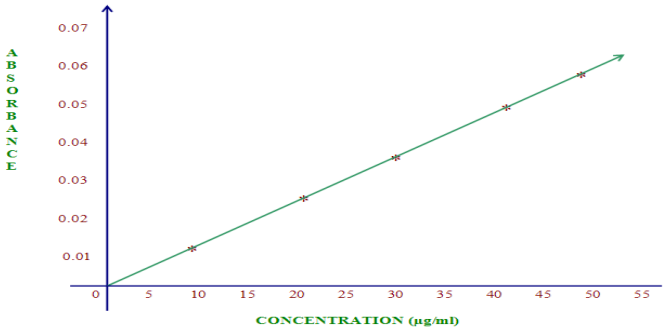


Fig:No:6

Linearity curve of Tribulus terrestris by doublebeam UV spectrophotometry



Tab.No 1
Moisture content.

S .No	Weight of drug taken	Weight after heating	Loss on drying
1	5 gm	3.73 gm	1.27 gm
2	5 gm	3.62 gm	1.38 gm
3	5 gm	3.80 gm	1.20 gm

Tab.No 2
Total ash

S .No	Weight of drug taken	weight of ash obtained
1	4 gm	1.20 gm
2	4 gm	1.10 gm
3	4 gm	1.00 gm

Tab.No 3
Acid insoluble ash

S .No	Weight of ash taken	Weight of residue
1	0.25 gm	0.04 gm
2	0.25 gm	0.03 gm
3	0.25 gm	0.06 gm

Tab.No 4
Proximate analysis of dry fruit powder of Tribulus terrestris Linn

S .No	Test	Percentage
1	Moisture content	25.66
2	Total ash	27.50
3	Acid insoluble ash	17.2

Tab.No 5
Nitrate content by double beam UV spectro photometer

Concentration (µg/ml)	Absorbance (nm)
0	0.000
10	0.011
20	0.025
30	0.039
40	0.050
50	0.059

Tab.No 6
Nitrate content by colorimetry

Concentration (µg/ml)	Absorbance (nm)
0	0.00
10	0.06
20	0.15
30	0.22
40	0.30
50	0.36

DISCUSSION

The results of proximate analysis of fruit powder of *Tribulus terrestris* Linn were compared with the reported values of proximate analysis. A comparison of methanolic extracts of fruits of *Tribulus terrestris* Linn leaf (Fig.No.1), fruit (Fig.No.2), and root powder (Fig.No.3) were done with whole plant, the whole plant powder extract was showing (Fig.No.4) HPTLC method indicating that the band with R_f value of 0.65 was observed in each developed and scanned chromatographic plate. The other bands present in *Tribulus terrestris* Linn methanolic extract of fruits, roots and leaves were found to be different and hence it is possible to differentiate plant parts of *Tribulus terrestris* Linn. A study of percentage composition of various phyto chemicals present in *Tribulus terrestris* Linn was performed. Nitrate content in fruit powder was showed linearity curve as shown in the (Tab.No.5 & Fig.no.5) using double beam UV spectrophotometry and colorimetry shown in (Tab.No.6 & Fig.no.6).

CONCLUSION

The results of proximate analysis of fruit

powder of *Tribulus terrestris* Linn were compared with the reported values of proximate analysis. A comparison of methanolic extracts of fruits of *Tribulus terrestris* Linn roots, fruits, and root powder were done with whole plant, the whole plant powder extract was showing HPTLC method indicating that the band with R_f value of 0.65 was observed in each developed and scanned chromatographic plate. The other bands present in *Tribulus terrestris* Linn methanolic extract of fruits, roots and leaves were found to be different and hence it is possible to differentiate plant parts of *Tribulus terrestris* Linn. A study of percentage composition of various phyto chemicals present in *Tribulus terrestris* Linn was performed. Nitrate content in fruit powder was showed linearity curve as shown in the figure using double beam UV spectrophotometry and colorimetry. The above parameters like ash value, nitrate content, moisture content and proximate analysis complies the WHO guidelines and limits.

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