



PHARMACOGNOSTICAL & PHYTOCHEMICAL EVALUATION OF *BRASSICA OLERACEA* LINN VAR. *CAPITATA* F. *RUBRA* (THE RED CABBAGE)

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ABSTRACT

The red cabbage (RC) (*Brassica oleracea* var. *capitata* f. *rubra*) belonging to the family Brassicaceae (order - Brassicales), is an herbaceous, biennial, dicotyledonous flowering plant. Red cabbage is a rich and relatively cheap source of anthocyanin pigment. The principle constituents of RC are isothiocyanates (glucosinolate), vitamins A, B, C and anthocyanins. The objective of the present study has been to standardize such a therapeutically useful plant, to avoid its adulteration or substitution. In the present study, the pharmacognostic standardization of powdered leaves of RC was carried out to determine its macro and microscopical characters and also some of its quantitative standards. Physico-chemical evaluation includes ash values, extractive values, moisture content was evaluated. Preliminary phytochemical screening of the aqueous extract of RC powder was carried out. This was further confirmed by carrying out thin layer chromatographic (TLC) studies. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the herb, which is gaining relevance in plant drug research.

Keywords: *Brassica oleracea*, Brassicaceae, Standardization, Leaves, TLC.

INTRODUCTION

Most of the modern researches on herbal medicine have hinged around traditional folklore medicine due to the fact that modern medicine has brought with it an array of drugs, none of which is nontoxic and quite safer for human consumption. The red cabbage (RC) (*Brassica oleracea* var. *capitata* f. *rubra*) belonging to the family Brassicaceae (order - Brassicales), is an herbaceous, biennial, dicotyledonous flowering plant (fig. 1). Its leaves are coloured dark red/purple. It can be found in Northern Europe, Northern America and China. Presence of flavonoids was reported in RC species and flavonoids have good therapeutic potential in inflammation and pain. Red cabbage is a rich and relatively cheap source of anthocyanin pigment. Its extract has a powerful coloring and a superior stability compared with other anthocyanins, because of its chemical configuration [1]. Sulfurated substances are co-extracted with the pigment producing a disagreeable odor [2]. Among myriad natural plants, RC and other Brassica vegetables, vegetables endemic to the Mediterranean

region, have been found to have antioxidant, antihyperglycemic [3-5], anticancer [6-8] and hypocholesterolemic [9] properties. RC extract has also prevented oxidative stress induced in livers and brains of animals exposed to paraquat [10] and N-methyl-D-aspartate [11]. The principle constituents of RC are isothiocyanates [glucosinolate], vitamins A, B, C and anthocyanins [8,12,13]. Anthocyanins, a group of phenolic natural pigments present in RC, were found to have the strongest antioxidant power of 150 flavonoids [14]. The objective of the present study has been to standardize such a therapeutically useful plant, to avoid its adulteration or substitution.

Materials and Methods

Plant material

Fresh RCs were purchased from a local market at Tirupati.

The leaves were sliced, dried under shade, powdered and passed through 40-mesh sieve.



Fig:1 Red cabbage

Macroscopy

Color: Purple

Taste: Bitter

Odour: Characteristic

Powder microscopy:

The microscopic powder analysis was done according to the method of Brain and Turner [15] and Kokate [16].

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values, extractive values, Fluorescence analysis and moisture content of powder sample of RC was performed according to the official methods prescribed [17] and the WHO guidelines on quality control methods for medicinal plant materials [18-20].

Extraction of RC

Aqueous extract was prepared by macerating the dried drug powder in double distilled water. The extract was concentrated in water bath and then used for phytochemical screening.

Preliminary phytochemical screening

Preliminary phytochemical screening of the above extract was carried out by using standard procedures described by Kokate [21] and Harborne [22].

Thin layer Chromatography

A thin layer of silica gel and slurry was coated on to the plates and it is evenly distributed. The plates are then air dried. TLC Plates are activated (in hot air oven at 120°C for 15-20 minutes) after 5-10 minutes of their preparation. This served as stationary phase. The mobile phase used were Hydrochloric acid, Formic acid and Water (1.9:3.9:4.1) [23] and N- butanol, Acetic acid and Water (4:1:5). The plates were observed for the spot and the R_f values (equation 1) were calculated and noted. Ammonia vapor was used as the derivatising agent, as the extract of RC is rich in flavonoids (anthocyanins).

The R_f value is calculated using the formula:

$$R_f = \frac{\text{Distance travelled by solute from the base line}}{\text{Distance travelled by solvent front}}$$

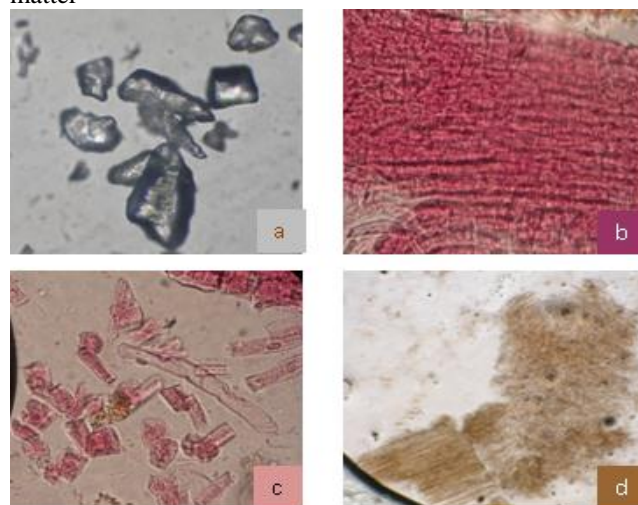
Equation 1

RESULTS AND DISCUSSION

Powder characters of RC

The RC powder consisted of the following elements when viewed under the microscope; Prism type Calcium Oxalate crystals were present (fig. 2a). Strongly lignified parenchymatous tissue whose cells are rectangular to square in shape was found (fig. 2b). Vessel elements of different sizes are frequently seen in the powder (fig. 2c). The vessel elements, which are wide, short and drum shaped and also long, narrow and cylindrical shape are present in the powder (fig. 2c). Brownish matter is also present throughout the tissue (fig. 2d).

Fig. 2. a. Prism Ca. Oxalate crystals; b. Lignified parenchymatous tissue; c. Vessel elements; d. Brownish matter



Physico-chemical parameters

The physico-chemical evaluation of a crude drug involves the determination of identity, purity and quality. Extractive values (Table 1) are useful for determination of crude drugs & it gives an idea about the nature of the chemical constituents present. Since the water soluble extractive value was found to be higher, this indicates that the concentration of polar compounds (eg., Flavonoids) may be high in RC. Ash value is a criterion to judge the identity and purity of crude drug. The ash values (Table 2) of the powdered RC shows a high concentration of sulphated ash. The results of fluorescence analysis, which is a standardizing parameter of the drug powder, are presented in Table 3. The percentage moisture content in the RC powder was found to be 10.0 %w/w.

Table 1. Extractive values of powdered RC

Extractive values	RC powder (%w/w)
Alcohol soluble extractive	5.40
Ether soluble extractive	0.60
Water soluble extractive	6.00

Table 2. Ash values of powdered RC

Ash values	RC powder (%w/w)
Total Ash value	8.90
Acid insoluble ash value	5.50
Water soluble ash value	2.00
Sulphated ash value	7.40

The chief phytochemicals present in the aqueous extract of *Brassica oleracea* var. capitata f. rubra were carbohydrates, proteins, glycosides, flavonoids, phenolic compounds and the results are tabulated (Table 4).

Thin Layer Chromatography

The R_f values of the spots obtained in the TLC studies of aqueous extract of *Brassica oleracea* var. capitata f. rubra is given in table 5 and the plates of TLC eluted in various mobile phases are shown in fig. 3a & 3b.

Table 4. Preliminary Phytochemical Analysis of aqueous extract of *Brassica oleracea* var. capitata f. rubra

Phytoconstituents	Aqueous extract of Red cabbage
Alkaloids	-
Carbohydrates	+
Proteins	+
Glycosides	+
Flavonoids	+
Phenolic compounds	+
Proteins	+

'+' indicates Presence; '-' indicates Absence

Table 5. R_f values of the spots obtained in the TLC studies of various phytoconstituents

Mobile phase	R_f value
Hcl, Formic acid & Water (1.9:3.9:4.1)	0.84
N-butanol, Acetic acid & Water (4:1:5)	0.76

Table 3. Fluorescence analysis of powdered RC

Treatments (RC powder)	Observations		
	Day light	Short UV (254 nm)	Long UV (365 nm)
Powder	Pale purple	Brown	Purple
Powder + 1N HNO ₃	Pale pink	Brown	Pink
Powder + 1N HCl	Pale pink	Brown	Pink
Powder + 1N NaOH (aqueous)	Yellow	Green	Pale Yellow
Powder + 1N NaOH (alcoholic)	Yellow	Green	Pale Yellow
Powder + Ammonia	Yellowish brown	Dark Brown	brown
Powder + Iodine	Blue	Dark blue	Blue

CONCLUSION

The present work was dealt with an intention to fix standards which could be useful to detect the authenticity of this medicinally useful plant. The sample of *Brassica oleracea* var. capitata f. rubra exhibits a set of diagnostic characters, which will help to identify the drug in dried condition. The taxonomical identification of plant

Fig 3a. T L C of Red cabbage Extract HCl, Formic acid & Water (1.9:3.9:4.1)**Fig 3b. T L C of Red cabbage Extract N-butanol, Acetic acid & Water (4:1:5)**

material and pharmacognostic evaluation is important to provide the standards and to avoid adulteration of drugs. Macroscopic and Microscopic characters of the plant are used for the identification of the drug. The physicochemical evaluation helps in formulating pharmacopoeial standards, while fluorescence analysis helps in distinguishing the drug in powder form. The

physico chemical constants like moisture content, ash values, extractive values and fluorescence analysis are rarely constant for crude drugs, but they may help in evaluation. Ash value is a criterion to judge the identity and purity of crude drug. The extract obtained by exhausting crude drug is indicative of approximate measure of their chemical constituents. Determination of the moisture content helps prevent degradation. The phytochemical analysis helps in chemoprofiling, which aids in determining the major therapeutically useful constituent present in the plant extracts. These phytochemicals were confirmed by using

chromatographic techniques like TLC. Standardization of a plant which has various therapeutic applications is a prerequisite; as such plants are very frequently prone to adulteration or substituted. The periodic assessment is essential for quality assurance and safer use of herbal drugs [24].

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