Research Article

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EVALUATION OF PHYSICO CHEMICAL PROPERTIES AND DIURETIC ACTIVITY OF BIOTITE CALX (ABHRAKA BHASMA) PROCESSED WITH *TRIBULUS TERRESTRIS* L.: AN EXPERIMENTAL STUDY

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ABSTRACT

Ayurvedic formulations have been used for centuries with claimed safety and efficacy. The aim of the present study was to evaluate the diuretic effect of Gokshura Kwatha Marita Abhraka Bhasma (biotite calx incinerated with the decoction of *Tribulus terrestris L.*) in experimental animals. The animals were randomly selected and divided into three groups, each comprising 8 animals. The diuretic activity of Abhraka Bhasma (biotite calx) was evaluated by determining volume, pH and electrolyte concentration of urine in male albino rats at a single dose of 4.5 mg for 200g body weight. Furosemide was used as reference standard and normal saline was used as normal control. Abhraka Bhasma treated rats have shown significant increase in the volume of urine and urinary electrolyte concentrations of Na^+, K^+ and Cl^- ions in comparison to normal control. Thus, these results indicate that Abhraka Bhasma processed with the decoction of *Tribulus terrestris L.* could have potent diuretic activity.

Key words: Abhraka Bhasma, Diuretic activity, Furosemide, Gokshura, Kwatha

INTRODUCTION

The approach of Ayurveda has been used to achieve optimum health as well as treatment of various diseases.[1]. Diuretics increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephrotic syndrome and cirrhosis. Even though several diuretic drugs are available in modern medicine, some of these have adverse effects such as hyponatremia, circulatory collapse, thromboembolic episodes, cardiac arrythmia, ototoxicity, hyperuricemia, hyperglycemia, parasthesias, and bone marrow depression and gastro intestinal disturbances etc [2, 3]. Although several diuretic drugs are successful in curing with adverse effects, there is an extended interest in using Kastaousadhis (Herbal formulations) as an alternative medicine to neutralize the side effects and cost effective. In Ayurvedic classics, biotite calx has been indicated in conditions such as mootra krichara (dysuria), mootra ghata (incontinence) [4]. *Tribulus terrestris* has been included among the herbs used for incineration/calcination of biotite[5] and it has been included under Mootra Virechaniya Gana Dravyas (group of herbs used to increase the flow of urine) in classics[6]. Hence, Ayurvedic pharmacopoeia refers *T. terrestris.* L. as Gokshura and it has been used as a source for various formulations[7]. *Tribulus terrestris L.*, commonly known as puncture vine, belongs to the family *Zygophyllaceae* and is widely distributed in both tropical and mild temperate regions[8]. Traditionally, *Tribulus terrestris L.* is a medicinal plant containing numerous steroidal saponins[9] and has also been reported for treatment of various ailments[10].The plant extract is used for urinary dysfunction[11, 12] and have also shown to have anti-hypertensive and vasodilatory properties[13]. It exhibits anti oxidant, antitumor, antifungal and anti-helmenthic properties [14-17]. These properties have created interest for studying the diuretic activity by underlying the exerting mechanism of *T. terrestris L.* for selecting it as a source. Thus considering the above factors, the present study has been designed to evaluate the physicochemical analysis and diuretic effect of biotite calx triturated with the decoction of *T. terrestris L.*

MATERIALS AND METHODS

Identification and characterization of raw materials

An important part in the preparation of medicament lies in proper identification and procurement of the raw materials. This determines the quality of the drug. Special request was made to the Mineralogists of Dharwad Mineralogy Department, Karnataka, K.U.D, for selecting the best quality Abhraka Bhasma. On the basis of their suggestion, Krishna Vajrabhraka (biotite) collected from Chennai was selected and implicated for Bhasmeekarana (calcination/incineration). For Abhraka Bhasma preparation medicinal plants like *Tribulus terrestris L.* and *Rubia cordifolia L.* have been collected from Kattankulathur, Kancheepuram district, Tamil Nadu, India respectively in December 2008. The plant (*Tribulus terrestris L.* and *Rubia cordifolia L.*) sample was identified and authenticated by Plant Anatomist and a voucher specimen (*Tribulus terrestris*;

ISISM/RES/B0107) and (*Rubia cordifolia*; ISISM/RES/B0558) has been preserved for future reference. The physicochemical characterization of *T. terrestris L*. was done using high performance liquid chromatography analysis (RP-HPLC, Shimadzu LC 2020, Shimadzu, Japan).

Abhraka Bhasma preparation

The Abhraka Bhasma has been prepared by following steps as cited in Figure1.

Shodhana of Abhraka (Purification or detoxification of biotite)

The Grahya Abhraka (Genuine biotite) may also contain few adulterants, foreign materials which may cause complications, so by considering all these facts the Ayurvedic Scholars have mentioned various Shodhana (purification) procedures. The heat resistance properties and stratified structure of Abhraka, made Acharyas (ancient scholars of Ayurveda) to follow mainly heat & dip procedure (nirvapana) for Abhraka Shodhana with Kanij (sour gruel), Gomutra (cow's urine), Godugdha (cow's milk) and Triphala Kwatha (Three myrobalans decoction) for seven times in each liquid media[4]. The main purpose of Shodhana is to remove the water soluble and fat soluble impurities and destruction of stratified structure of Abhraka by converting it into a granular form especially before Marana (incineration/calcination).

Dhanyabhraka (Particle size reduction)

Dhanya (paddy) along with Shoditha Ahraka (purified biotite) was kept in a Jute bag and Pottali (bundle) was prepared and kept in Kanji. Probably interaction between liquid Kanji and solid Abhraka may take place resulting in reduction of particle size of the purified Abhraka[18].

Marana of Abhraka (Incineration/calcination)

The process of Marana (Incineration) was adopted to convert the heterogenous material into homogenous nanoparticles. In the present study Gajaputa (quantum of heat) was adopted, which exerts up to 1000˚C. Acharyas explained about different number of Putas (quantum of heat) for Abhraka Bhasmeekarana (calcination). In this process, Puta was given until "Bhasma Pariksha" (test for perfect calx) gets full filled. After 20th Puta, all "Bhasma Parikshas" was attained[18]. Amrutikarana was done to remove the doshas (residual) ill effects and then Lohiti karana (coloration) was carried out by giving 5 gajaputa with decoction of *R. cordifolia* to regain the brick red color (Ishtika Varna) of biotite calx. Puta can be decided on the basis of the dimensions of the raw drug and temperature.

Amruteekarana and Lohitikarana of Abhraka Bhasma

Amrutikarana was done to remove the doshas ill effects and then Lohiti karana was carried out by giving 5 gajaputa (1000˚C) with decoction of Manjista Kwatha (*R. cordifolia*) to regain the brick red colour of biotite calx [4,18].

Analytical study of Abhraka Bhasma

pH, Loss of drying, moisture content, Ash value, water soluble, acid soluble, and elemental or chemical characterization of a sample was carried for out using X-ray diffraction measurements (SMART lab, Rikagu, JAPAN).

***In vivo* study of Abhraka Bhasma in diuretic activity**
Experimental animals

Male albino rats weighing about 180 ± 20gm on average were obtained for experimental study. The animals were housed in standard conditions of controlled ambient temperature (22–24˚C), humidity (50 ± 15%) with a 12-h light/ dark cycle. Commercial pellet diet (Ratan brothers, India) and water were provided *ad libitum*. The experimental protocol was approved by the institutional animal ethical committee, Shri DGM Ayurvedic Medical College Hospital & PG Research Centre, Gadag, Karnataka, India.

Dose selection and schedule

In the classical texts[19], dose of Abhraka bhasma is mentioned as 2 Ratti (i.e. 250 mg for an adult). Considering this, the dose of the experimental animals was calculated by extrapolating the human dose to animals as 4.5 mg for 200g male albino rats.

Experimental design

The diuretic activity was determined by following the procedure as described previously with minor modifications [20]. The experimental animals were randomly divided into 3 groups each consisted of 8 male albino rats. The Group-I was kept as vehicle control, whereas the Group-II was administered with furosemide at a dose of 20mg/kg/b.w (body weight) and was used as a standard diuretic agent and Group-III were administered with Abhraka bhasma at a dose of 4.5 mg. The Abhraka Bhasma, furosemide and vehicles were administered to the overnight fasted rats of the respective groups. As the normal urine output in rats is very low (1-2 mL/rat/day), to get a measurable quantity of urine, the rats of all the groups were administered distilled water (2mL/100gm) after 30min of test drug administration. Then, the animals were placed individually in metabolic cages with netted floor and urine was collected in conical flasks placed below the polythene funnel of the metabolic cages. Extreme care was taken to avoid the contamination of urine with faecal matter. Urine output was observed, collected and recorded hourly. At the end of 2nd, 4th, 6th and 8th hours urine was collected and measured and the pH of each group of animals urine were determined [20, 21].Then animals were taken out of the cages and the total volume of urine excreted by each group was noted[22, 23].

Urine output analysis by flame photometer

The urine was collected after drug administration up to the fifth hour. The urine volume was measured and analyzed for Na^+, K^+ (cations), and Cl^- (anions). The concentration of Na^+, K^+, and $Cl^-$was analyzed with the help of a flame photometer [24, 25] and the amount of chloride was determined titrimetrically by silver nitrite solution (0.1 N), using one drop of 5% ferric alum solution as an indicator. The pH of urine was also measured using standard pH paper.

Statistical analysis

The data were statistically evaluated using GraphPad prism (Windows version 5.01). Values were presented as mean ± S.D of the three replicates of each experiment were separated by the unpaired Student's' test to determine significant differences in all parameters. p values <0.05 were considered to be statistically significant.

RESULTS

Analytical Study

Tribulus terrestris L. was selected for triturating Abhraka in order to enhance the diuretic effect of Abhraka bhasma, hence, *Tribulus terrestris L*. was subjected to HPLC to find out the presence of biomarkers like quercetin, rutin, ellagic acid and gallic acid which could be due its synergistic pharmacological activities. The reference standards like quercetin, rutin and ellagic acid were detected at 254 nm and gallic acid at 280 nm (Figure 2). The presence of quercetin, rutin, ellagic acid and gallic acid was confirmed by comparing chromatographic peaks in hydro-alcoholic extract of *Tribulus terrestris L*. with the retention time (Rt). The analytical study shown the following observations such as pH of 7.83, Loss on drying (at 110˚C) of 0.06% moisture which was negligible, total ash of 99.49% which implies the organic constituents and the left 0.51% in Abhraka Bhasma was in the inorganic form, acid insoluble ash of 90.88%, fineness of particle was of 125µ which was fine in nature. It was sparingly soluble in water and insoluble in chloroform. The quantitative estimation of Fe, Ca, Mg, Al_2O_3,Si in Abhraka Bhasma were 10.5%, 2.88%, 1.24%, 2.3% and 26.42% respectively.

***In vivo* study diuretic activity of Abhraka Bhasma**

The urine volume was expressed in mL and the concentrations of the electrolyte were expressed in mEq/L. The values obtained for the parameters in case of test drug (Abhraka Bhasma, 4.5mg for 200g body weight) were compared with that of standard drug (furosemide 20mg/kg/b.w) and control group by using unpaired student 't' test. The urine volume of rats at different time intervals in the control, furosemide and Abhraka Bhasma treated groups were shown in Figure 3a. The present study revealed that Abhraka Bhasma rats showed a significant increase ($p < 0.05$) in the urine volume as well as electrolyte concentrations of the urine when compared to the control group. The electrolytes (Na^+, K^+ and Cl^-) loss in Abhraka Bhasma was minimum when compared to the standard group (Figure 3b). This shows that there is less chance of electrolyte imbalance in the Abhraka Bhasma treated group. Similarly no significant change was observed in pH of the urine in all groups. These results indicate that Abhraka Bhasma processed with Gokshura Kwatha possess good diuretic activity.

DISCUSSION

Abhraka Bhasma has been mentioned as Sarvarogahara (remedy for all the diseases) which describes its broad-spectrum therapeutic uses. It alleviates majority of physical ailments with suitable combination / drugs[18]. In experimental study, diuretic effect of the orally administered Abhraka Bhasma was evaluated in male albino rats and compared with furosemide widely used in clinical practice. The main two components for diuresis are increase in urine volume and loss of electrolytes in urine. These processes may be due to suppression of renal tubular re-absorption of water and electrolytes into the blood stream. The urine volume concentrations of electrolytes in the urine such as sodium, potassium and chloride were the parameters measured while assessing the diuretic potential of the drug. Thiazides act in sodium level by decreasing the sodium re-absorption in the distal convoluted tubule. This occurs due to the inhibition of the Na^+/Cl^- co-transporter on the luminal membrane[26]. There is increased loss of K^+ ion due to electrolyte exchange of K^+ for Na^+ in the collecting duct and there was no significant increase in urinary Cl^- level in urine. Hence it may be assumed that test drug (Abhraka Bhasma) increases urine output and urinary excretion of sodium by inhibiting (Na^+, K^+, Cl^-) co-transporter system in the thick ascending limb of the Loop of Henley.It can be suggested that the test drug (Abhraka Bhasma) may also have similar type of action like Thiazides to produce diuretic activity. Further pharmacodynamic & kinetic studies are required to assess the clinical efficacy of the test drug as a potential diuretic agent.

CONCLUSION

The experimental study reveals that Abhraka Bhasma (biotite calx) processed with decoction of Gokshura Kwatha (*Tribulus terrestris.* L) increased urinary volume and electrolyte (sodium and potassium) excretion. The mode of action Abhraka Bhasma was similar to that of the reference drug (furosemide), suggesting a similar mechanism of action. Thus, this study suggests that Abhraka Bhasma processed with the decoction of *T. terrestris L*. might be a potential alternative drug for diuretic activity.

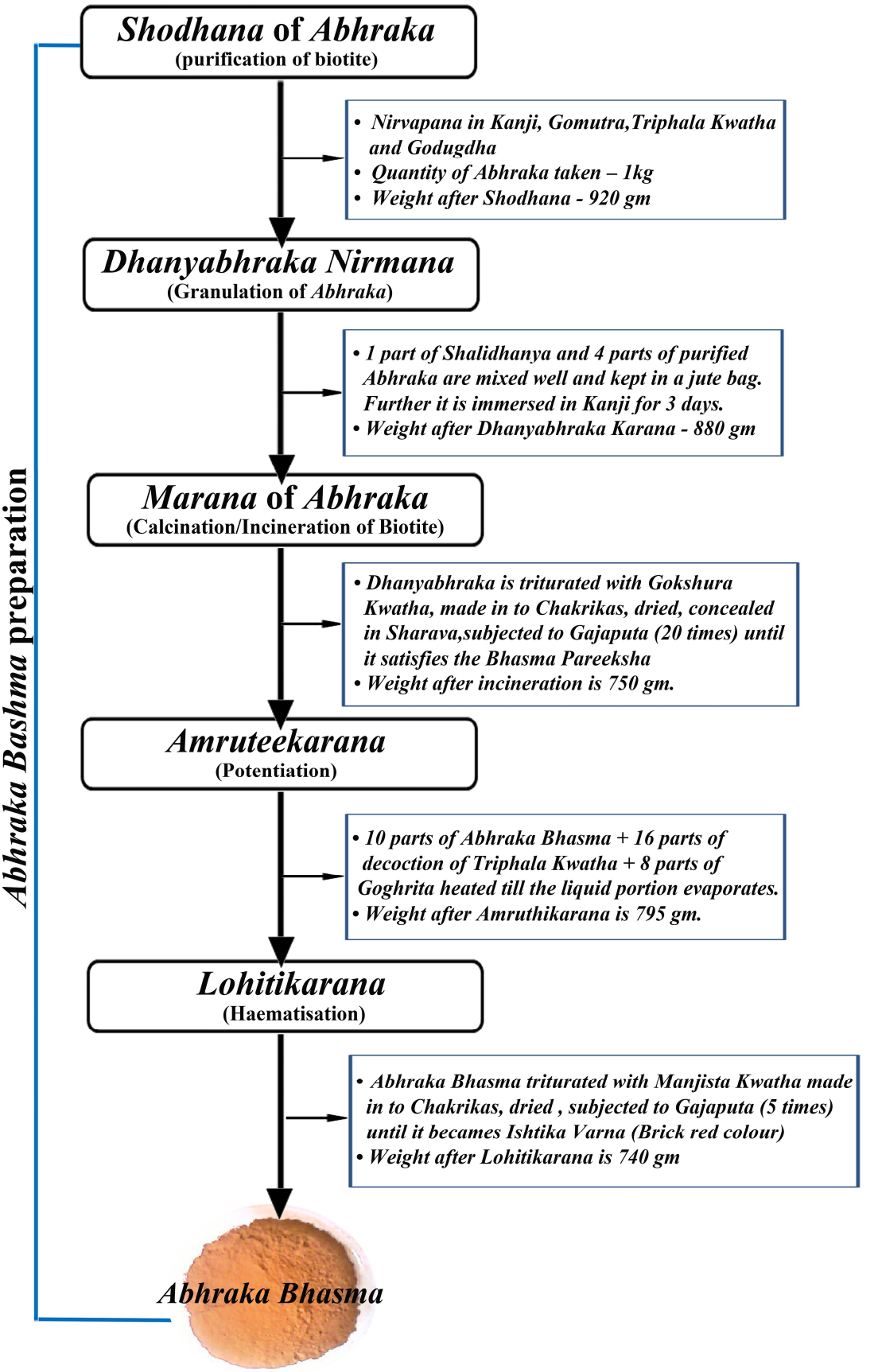


Figure 1: Abhraka Bhasma preparation

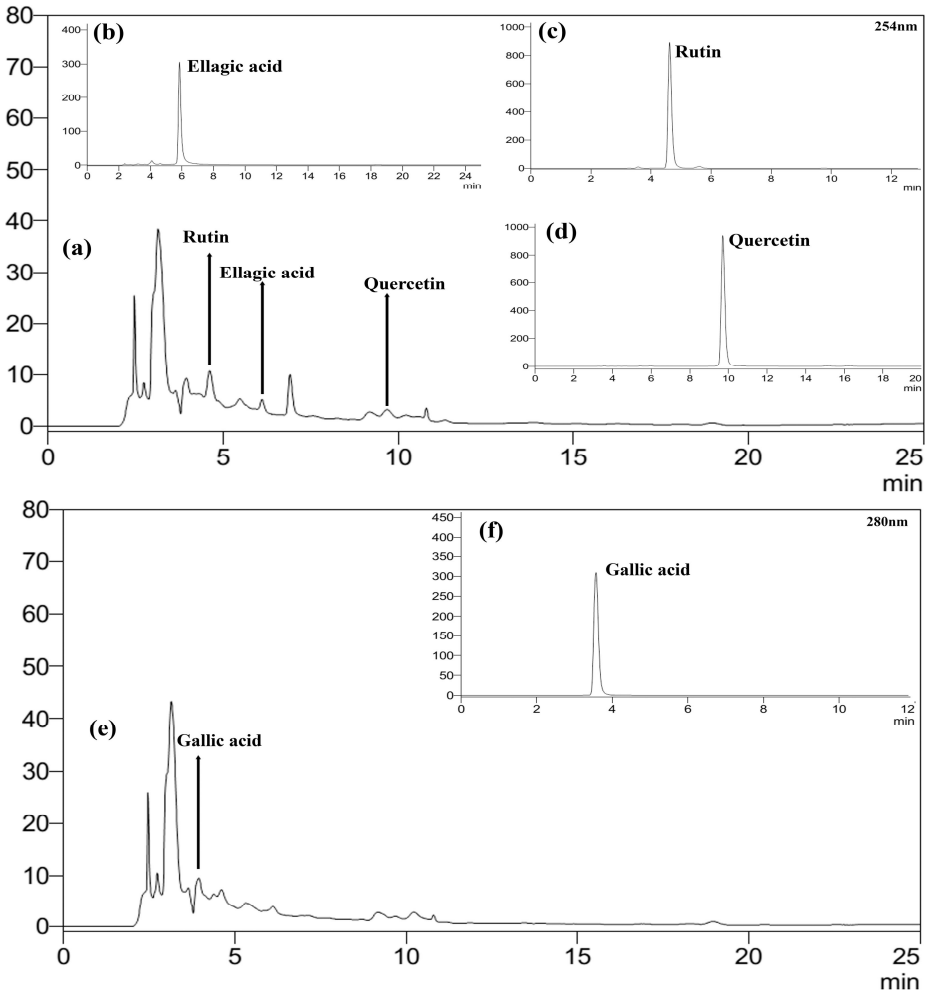
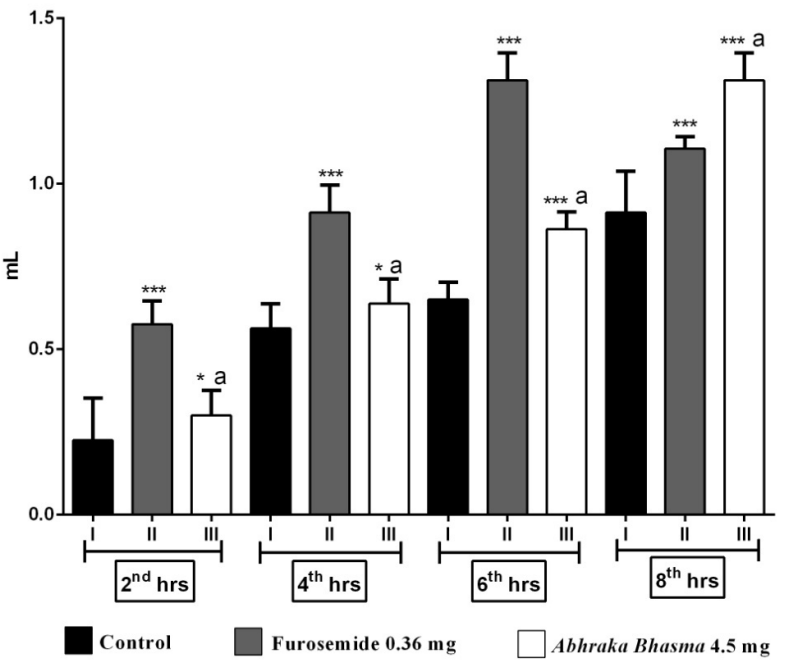
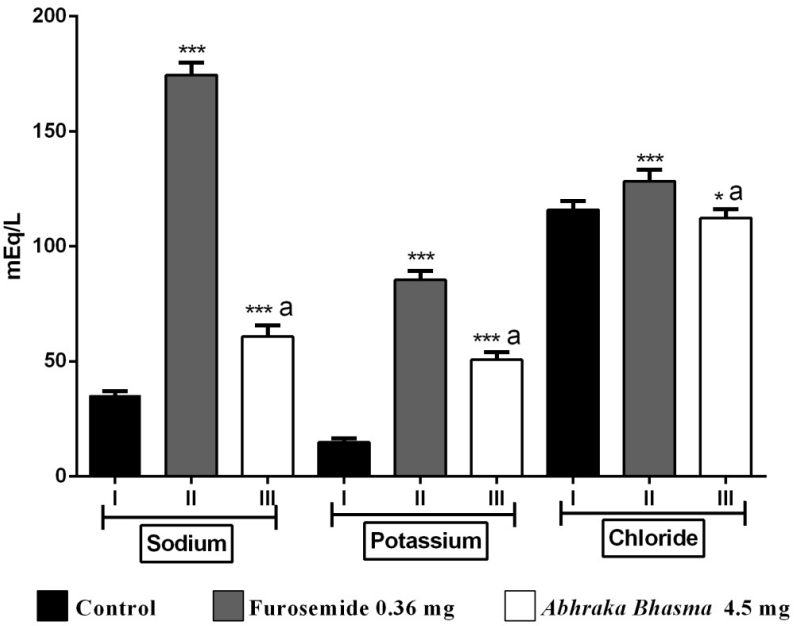


Figure 2: Qualitative HPLC chromatograms: (a) hydro-alcoholic extract of *Tribulus terrestris* at 254nm and (e) at 280nm, (inset standard marker compound (b) ellagic acid, (c) rutin, (d) quercetin and (f) gallic acid.)



(a)



(b)

Figure 3: (a) Effect of Abhraka Bhasma on urine output in rats at different time intervals. (b) Effect of Abhraka Bhasma on urine electrolyte in rats. Data are expressed as mean ± S.D. (n = 8 rats) unpaired 't' test;. Significant differences in each group vs control are *P<0.05, **P<0.01, ***P<0.001; a = P<0.001 were standard vs trial group.

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