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COMPOSITIONAL CHARACTERISTICS OF RUDRAKSHA *(ELAEOCARPUS GANITRUS Roxb.)*

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ABSTRACT

Rudraksha bead or *Elaeocarpus ganitrus* (Roxb.) is a reverent plant based therapeutic agent with spiritual and holistic importance in traditional system of medicine since time immemorial. While multifarious pharmacological properties of Rudraksha beads have been reported against a range of chronic disorders, evidences about its compositional characteristics are highly superficial. The present study aims to quantify the biochemical, phytochemicals, functional groups, and elemental characteristics of three, four, and five Mukhi Rudraksha (3MR, 4MR, and 5MR) beads. Biochemical analysis encompasses quantification of total carbohydrates, total proteins, and crude fat content. Phytochemical analysis was performed to quantify alkaloids, saponins, flavonoids, phenolics, anthocyanins, ascorbic acid, tannins, and terpenoids. Identification of functional groups was carried out through Fourier transform infrared (FTIR) spectrophotometer and UV spectrophotometer. Elemental analysis was performed using CHNSO analyzer. One Way ANOVA with Posthoc Multiple Tukeys test was used for statistical analysis. Significant differences were obtained in all the biochemical, phytochemicals, functional groups, and elemental components among 3MR, 4MR, and 5MR. Future studies are warranted to identify the active biomarkers in different Mukhi Rudraksha that will add new therapeutic candidates in the ever-growing number.

Keywords: Rudraksha, biochemical, phytochemical, elemental, traditional system of medicine, physical, physicochemical, functional groups

INTRODUCTION

Rudraksha bead or *Elaeocarpus ganitrus* (Roxb.) is a reverent plant based therapeutic agent with spiritual and holistic importance in traditional system of medicine since time immemorial (Joshi and Jain, 2014; Naresh *et al.,* 2003). Being a member of the Elaeocarpaceae family, around 360 species of Elaeocarpus have been reported worldwide, out of which 25 species are found in India (Gangetic plains and Himalayan regions) (Joshi and Jain, 2014). Further, 21 types of Rudraksha bead are available that are categorized based upon the presence of the number of natural grooves (thin vertical lines) or Mukhi's present on its surface (Hardainiyan *et al.,* 2015).

Multifarious pharmacological properties of Rudraksha beads have been reported against chronic disorders of nervous system, cardiovascular, respiratory, and digestive system, both collectively as well as Mukhi wise based upon the traditional literature (Singh *et al.,* 2010; Nain *et al.,* 2012; Sakat *et al.,* 2009; Shah *et al.,* 2010; Jawla and Rai, 2016). However, a huge gap exists related to the biochemical, phytochemicals, functional group and elemental characteristics of Rudraksha beads majorly due to the unavailability of quantitative data. Few studies documented the qualitative presence of major bioactive components (glycosides, alkaloids, vitamins, steroids, flavanoid, gallic acid, ellagic acid, quercetin, phytosterols, carbohydrate, tannin, flavonoid, amino acid, saponin, and terpenoids) in Rudraksha beads in different solvents with no quantitative analysis (Jawla and Rai, 2016; Dalei and Sahoo, 2016; Tripathi *et al.,* 2015; Das *et al.,* 2017; Tripathy *et al.,* 2016). Considering the importance of compositional characteristics in understanding the therapeutic properties of natural products and the presence of existing research gaps in the quantitative analysis of Rudraksha beads, the present study aims to evaluate the compositional (biochemical, phytochemicals, functional groups, and elemental) characteristics of three, four, and five Mukhi Rudraksha (3MR, 4MR, and 5MR) beads present in abundance in nature. It is believed that the study outcomes will add significant scientific evidences about the Rudraksha beads composition and Mukhi wise variations, if any.

MATERIALS AND METHODS

Sample Procurement and Preparation:

Rudraksha beads were collected from the repository of Kunwar Shekhar Vijendra Ayurvedic Medical College and Research Center, Shobhit University, Gangoh. Beads were subjected to a high-pressure air blower followed by thorough rinsing under running tap water and double distilled water to remove adhering dust particles and shade dried under controlled conditions further identified for specific Mukhi. The dried beads were then grounded in the mixer and sieved using a sieve shaker to obtain uniform powder.

Biochemical Analysis

Biochemical analysis was conducted to estimate total carbohydrates, total proteins, and crude fat in Rudraksha beads. Total carbohydrate content was carried out using the method explained by Hedge and his associates (Hedge *et al.,* 1962). Briefly, 2g of the powdered sample was hydrolyzed for three hours in a boiling water bath in presence of 5.0 ml of 2.5 N HCl followed by neutralization using sodium carbonate. The sample was centrifuged and appropriately diluted supernatant (1.0 mL) was mixed with 4.0 ml of anthrone reagent. The resultant samples were heated for eight minutes in a boiling water bath, cooled rapidly and the change in color from green to dark green was read at 630 nm against glucose standard (Hedge *et al.,* 1962). Total protein content was estimated using Lowry's method as described previously (Lowry *et al.,* 1951). Crude fat was evaluated using the method proposed by Sadasivam, 1996. This method is based on the weight difference of the sample before and after the petroleum ether extraction (Sadasivam, 1996).

Phytochemical Analysis

Defatted Rudraksha beads powder overnight macerated in methanol or ethanol as per the requirement of procedure to assess several phytochemicals including ascorbic acid, flavonoid, anthocyanin, phenolic, saponin, terpenoid, and alkaloid. Total flavonoid content was measured using the aluminium chloride colorimetric assay method. Briefly, 1 ml of methanolic extract was mixed with 4 ml of distilled water and 0.30 ml of 5 % sodium nitrite. After 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed and again kept for 5 minutes incubation. The solution was further treated with 2 ml of 1M sodium hydroxide and diluted up to 10 ml with distilled water for absorbance reading at 510 nm against reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) (Bohnr and Kocipai, 1994) Phenolic content was analysed using Folin-Ciocalteu assay. One ml of ethanolic extract of Rudraksha beads was mixed with 9 ml of distilled water and 1 mL of Folin-Ciocalteu phenol reagent and vortexed vigorously. After 5 minutes, 10 ml of 7 % sodium carbonate solution was added to the mixture and total reaction mixture volume was made up to 25 ml by adding distilled water. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner and incubated for 90 min at room temperature and then absorbance was determined against the reagent blank at 550 nm (Saxena *et al.,* 2012). Tannins was also determined by Folin - Ciocalteu method where 0.1 ml of methanolic sample extract was mixed 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35 % Na_2CO_3 solution. The reaction mixture was made up to 10 ml by adding distilled water and rigorously vortexed followed by 30 min incubation at room temperature. Tannins content was measured at 725 nm against set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) (Mohammed and Manan, 2015). Alkaloid content was measured in the methanolic extract of plant sample using acid – base titration followed by chloroform extraction as described elsewhere (Harborne, 1973). Ascorbic acid and anthocyanin content were estimated using the method described by Omaye *et al.,* (1979) and pH differential spectroscopic method respectively (Cheng and Breen, 1991). Saponin and terpenoid content were determined by double extraction gravimetric method as elaborated by Harborne, (1973).

Functional group Analysis

Identification of functional groups was carried out through Fourier transform infrared (FTIR) spectrophotometer and UV spectrophotometer. For FT-IR analysis, Rudraksha bead powder was mixed with KBr in the ratio of 1:8 with the help of mortar pestle and converted into 13mm pellets using pelletizing die and hydraulic pressing at 3000 psi pressure. The pellets were kept into solid sample holder and the spectrum was evaluated for full wavelength range in pre-calibrated FT-IR (700-4000 cm^{-1}) at room temperature using Fourier-transform infrared (FT-IR) spectroscopy (Agilent Cary 630, Perkin Elmer). After blank subtraction the remaining absorption spectra was compared with the reference absorption peak in in-built library available for the active functional group.

Samples were also evaluated at photo-luminance mode for the full range of the spectrum 190-1100 nm available with UV Spectrophotometer (Shimadzu-1800). The absorption for the solvent was automatically subtracted from the solution and maximum absorption peaks was recorded.

Elemental Analysis

Carbon, hydrogen, nitrogen and oxygen (CHNO) analysis of powdered Rudraksha beads were carried out using Thermo Scientific (FLASH 2000) CHN Elemental Analyser and data obtained was processed by the Eager 300 software.

Statistical Analysis

All results presented as Mean ± SD. Statistical data analysis was performed using Graph Prism Pad Software version 8.0 and $P<0.05$ was considered as statistically significant. The intergroup variation was measured by one-way ANOVA analysis followed by Post hoc Tukey test.

RESULTS

The detailed research outcomes of the basic physical, physicochemical, biochemical, phytochemicals, functional groups, and elemental characteristics of 3MR, 4MR, and 5MR beads are presented.

Biochemical characteristics of 3MR, 4MR, and 5MR

Table 1 represented the quantitative outcomes of the three basic biochemical compounds namely crude fat, total protein and total carbohydrates. All the three components were present in equivalent concentrations in 3MR, 4MwR, and 5MR and no statistical differences were obtained in any case.

Phytochemical characteristics of 3MR, 4MR, and 5MR

Table 2 demonstrated the outcomes of phytochemical analysis indicating the presence of flavonoids, tannins, phenolics, anthocyanin, ascorbic acid, saponins, alkaloids, and terpenoids in all three Mukhi's of Rudraksha (3MR, 4MR and 5MR). Concentration of flavonoids, total phenols, and terpenoids were found to be maximum in 5MR followed by 3MR and 4MR with significant Mukhi wise variations ($P<0.05$). Similarly, concentration of anthocyanins and alkaloids was also noticed to be maximum in 5MR followed by 4MR and 3MR with significant Mukhi wise variations ($P<0.05$). Four Mukhi Rudraksha possess maximum concentration of ascorbic acid and tannins with significantly different variations from other Mukhi Rudraksha ($P<0.05$), however the further order differed for these components. While ascorbic acid was found in the order of 4MR>3MR>5MR, tannins was found in the order of 4MR>5MR>3MR. Saponins was the only phytochemical component present in maximum concentration in 3MR followed by 5MR and 4MR with significant inter Mukhi variations ($P<0.05$).

Functional group characteristics of 3MR, 4MR, and 5MR

Functional group analysis was performed through FTIR spectroscopy and UV-VIS analysis. Results of FTIR spectroscopy demonstrated the presence of alcohol, phenol, alkanes, carbonyl, aryl ketone, aromatic, alkenes, acid, and ether among different Mukhi Rudraksha (Table 3). Figure 1 a-c demonstrated the peaks detected in 3MR, 4MR and 5MR Rudraksha samples respectively. Rudraksha powder exhibited strong, broad, stretch H bonded absorption spectra at 3400 cm^{-1} that indicated O-H group. Presence of strong peak at 2900 cm^{-1} confirmed C-H group. A strong and stretched characteristic band at 1725 cm^{-1} indicated the presence of pair of carbonyls (C=O) group. Stretch band at 1685 cm^{-1} reflected aryl ketone group, medium-weak-multiple bands at 1490 cm^{-1}, strong stretch two consecutive bands at 1350, cm^{-1} and 1380 cm^{-1}, strong stretch band at 1225 cm^{-1} and 1100 cm^{-1} showed the presence of aromatic, nitrogen, acid and cyclic ether compounds respectively. Overall, the absorption spectrum was almost same for all three Mukhi's of Rudraksha and the absorption peaks were identified at same wavelength and the corresponding wavelength in numerical form is illustrated in Figure 1d.

Outcomes of UV-Vis analysis is illustrated in Table 4. All selected Rudraksha powder samples dissolved in water showed absorbance in the ultraviolet band of 200-400 nm only with no appearance of absorption peak in the visible spectrum band (400 nm-800 nm). The profile showed the peaks at 256nm, 287.5nm, 311.5nm, and 335.5 nm in all Rudraksha samples.

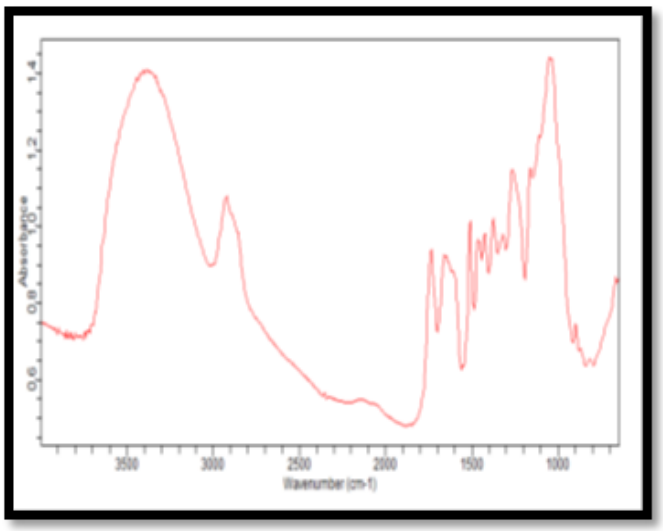
Elemental characteristics of 3MR, 4MR, and 5MR

Elemental analysis outcomes encompassing carbon, hydrogen, nitrogen and oxygen is demonstrated in Table 5. Percent carbon content in 3MR was lowest among all Mukhi Rudraksha and was significantly different when compared with 5MR ($P<0.05$). Similar to percent carbon content, percent nitrogen and hydrogen levels were also lowest in 3MR and was significantly different with both 4MR and 5MR ($P<0.05$). However, in case of oxygen levels an opposite trend was noticed with maximum percent oxygen content in 3MR with significant differences from 4MR and 5MR ($P<0.05$). The results of C/N ratio showed an obvious maximum value in 3MR when compared with 4MR and 5MR ($P<0.05$).

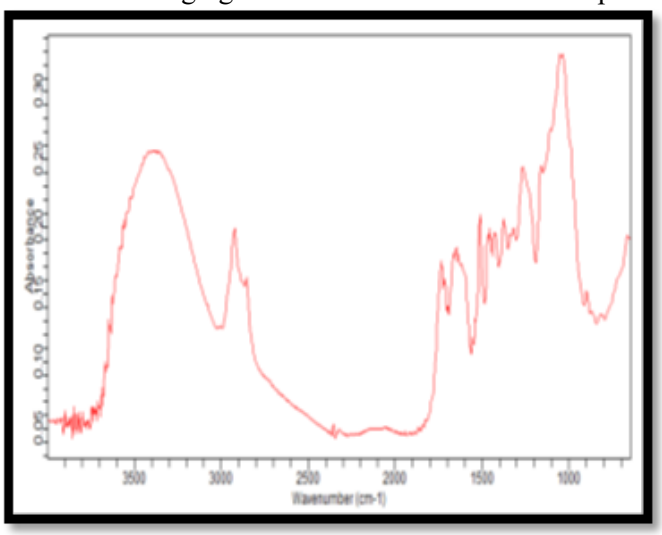
DISCUSSION

The present research provided comprehensive evidences about the basic physical, physicochemical, biochemical, phytochemicals, functional group, and elemental characteristics of three, four, and five Mukhi Rudraksha beads. The present study is the first to document quantitative outcomes of physicochemical, biochemical, phytochemical, and elemental components in selected Rudraksha to the best of our knowledge.

Biochemical analysis demonstrated the equivalent concentrations of total protein, total carbohydrate and crude fat content in 3MR, 4MR, and 5MR with no significant statistical differences. Previous studies revealed the qualitative presence of proteins and carbohydrates in different solvents extracts of random Rudraksha beads without Mukhi-wise segregation. While three studies reported



(a)



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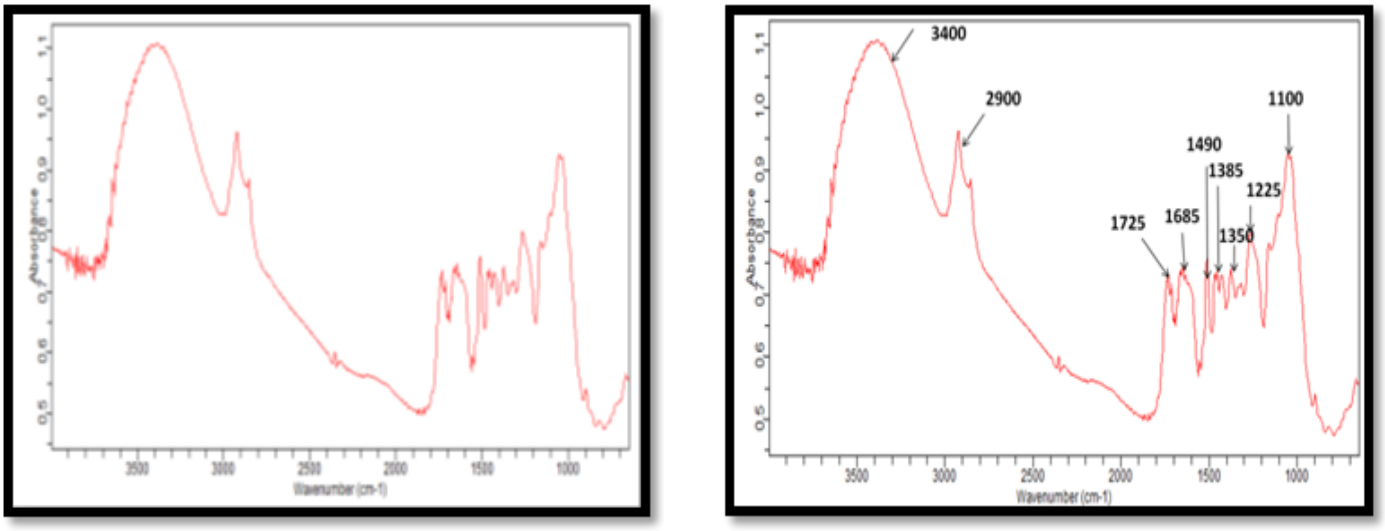

Figure 1 a-d: Representative FTIR Absorption Spectra of Rudraksha: Spikes are indicating absorption at particular wavelength for 3-5 Mukhi Rudraksha
(a) Absorption spectra of FTIR for 3 Mukhi Rudraksha **(b)** Absorption spectra of FTIR for 4 Mukhi Rudraksha **(c)** Absorption spectra of FTIR for 5 Mukhi Rudraksha **(d)** Representation of the corresponding absorption wavelength in graph.

Table 1: Results for biochemical characteristics of 3MR, 4MR, and 5MR

Parameters	3 MR	4 MR	5 MR	P value
Total protein (mg/g)	55.1 ±9.78	54.1 ±9.29	45.83 ±10.14	0.2281
Total Carbohydrate (mg/g)	52.10 ±3.62	51.46 ±2.88	51.19 ±4.32	0.9077
Crude Fat (mg/g)	191.33 ±10.23	172.5 ±21.37	184.6 ±6.56	0.0986

Data representing mean ± SD of six samples for each Mukhi Rudraksha. Statistical significant changes were obtained using One Way ANOVA and Posthoc Tukey test. Results represented no significant variations among the measured variables.

Table 2: Results for Phytochemical analysis of 3MR, 4MR, and 5MR

Parameters	3 MR	4 MR	5 MR	P value
Flavonoid (mg QE/g dry wt.)	0.89 ±0.076@	0.11 ±0.004*	1.53 ±0.22#	<0.0001
Tannin (mg QE/g dry wt.)	0.44 ±0.048@	0.71 ±0.07*	0.57 ±0.10#	<0.0001
Phenolic (mg GAE/g dry wt.)	0.74 ±0.20@	0.17 ±0.15*	0.88 ±0.09#	<0.0001
Anthocyanin (mg CYE/g dry wt.)	0.38 ±0.013@	0.50 ±0.046*	0.56 ±0.023#	<0.0001
Ascorbic Acid (mg AE /g dry wt.)	3.17 ±0.289@	4.52 ±0.385*	3.58 ±0.256#	<0.0001
Saponin (mg DIOE/g dry wt.)	11.83 ±2.15@	3.94 ±1.14*	5.55 ±0.86#	<0.0001
Alkaloid (mg ATROPE/g dry wt.)	9.05 ±1.61@	9.75 ±1.25*	17.44 ±1.80#	<0.0001
Terpenoid (mg LE/g dry wt.)	8.49 ±0.58@	7.24 ±0.43*	9.33 ±0.40#	<0.0001

*Data representing mean ± SD of six samples for each Mukhi Rudraksha. Statistically significant changes were obtained using One Way ANOVA and Posthoc Tukey test. Inter-group comparisons were represented as *P<0.05 3MR Vs 4MR; # P<0.05 4MR Vs 5MR; @P<0.05 5MR Vs 3MR. Note: phytochemicals concentration expressed as flavonoid expressed as mg quercetin equivalent (QE)/g, phenolic content as mg gallic acid equivalents GAE/g , tannin as mg quercetin equivalent (QE)/g, anthocyanin as mg cyanidin-3-glucoside equivalent (CYE)/g , ascorbic acid as mg ascorbate equivalent (AE)/g, saponin as mg diosgenin equivalent (DIOE)/g, Alkaloid mg atropine equivalent (ATROPE)/g, Terpenoid mg linalool equivalent (LE)/g of dried Rudraksha powder.*

Table 3: Results for Functional Groups Analysis of 3MR, 4MR, and 5MR

Peak Range	Reference Range	Functional Group	Compound	3 MR	4 MR	5 MR
3400 cm -1	3200-3600 cm -1	O-H (Alcohol, Phenol)	Poly Hydroxyl	+	+	+
2900 cm -1	2850-3000 cm -1	C-H	Alkanes	+	+	+
1725 cm -1	1720-1740 cm -1	C=O Carbonyl	Carbonyl	+	+	+
1685 cm -1	1680-1700 cm -1	C=O	Aryl Ketone	+	+	+
1600 cm -1	1600 cm -1	N-H	Amine	+	+	+
1490 cm -1	1400-1600 cm -1	C=C	Aromatic	+	+	+
1350, cm -1 1380 cm -1	1345-1385 cm -1	N-O	Nitro	+	+	+
1225 cm -1	1210-1320 cm -1	C-O	Acid	+	+	+
1150 cm -1	1080-1360 cm -1	C-N	Amine	+	+	+
1100 cm -1	1000-1150 cm -1	C-O-C	Cyclic Ether	+	+	+

Results for FTIR analysis: Positive sign (+) indicates the presence of specific groups in 3MR, 4MR and 5MR. All samples were analyzed in triplicates.

Table 4: UV-VIS absorption wavelength of 3MR, 4MR, and 5MR

Wavelength (λ in nm)	3 MR Absorbance	4 MR Absorbance	5 MR Absorbance
256	0.601	0.9946	0.6049
287.5	2.7111	3.0609	2.7222
311.5	2.6779	2.9702	2.6903
335.5	0.142	0.3805	0.1545

Maximum absorbance peaks at particular wavelength for 3MR, 4MR and 5MR. All samples were analyzed in triplicates

Table 5: Results for Elemental Analysis of 3MR, 4MR, and 5MR

Component	3 MR	4 MR	5 MR	P value
Carbon (%)	44.62 ±1.78@	45.88 ±4.57	49.04 ±1.18	0.0843
Nitrogen (%)	1.09 ±0.047@	1.90 ±0.11*	1.9 ±0.09	0.0011
Hydrogen (%)	4.51 ±0.013@	6.09 ±0.61*	6.53 ±0.46	0.0017
Oxygen (%)	35.49 ±1.01@	28.41 ±1.63*	28.01 ±0.73	0.0001
C/N	40.93 ±9.21@	24.18 ±2.31*	25.87 ±1.66	0.0005

*Data representing mean ± SD of six samples for each Mukhi Rudraksha. Statistical significant changes were obtained using One Way ANOVA and Posthoc Tukey test. Inter-group comparisons were represented as *P<0.05 3MR Vs 4MR; # P<0.05 4MR Vs 5MR; @P<0.05 MR Vs 3MR.*

absence and presence of total carbohydrates and total proteins respectively in methanolic extract of Rudraksha beads, Hardainiyan *et al.,* (2015) reported opposite results in ethanolic extract. Contradictory findings were reported in case of acetone extract where Dalei & Sahoo, (2016) documented absence of total carbohydrates and total proteins while Tripathi *et al*., (2015) documented presence of both components. Additionally, Tripathi *et al.,* (2015) also reported the presence of both these biochemical components in chloroform extract while both were absent in hexane and ethyl acetate extracts. Further, none of the previous studies reported the presence of crude fats in Rudraksha beads both qualitatively or quantitatively.

Quantitative phytochemical analysis demonstrated significant Mukhi wise variations in flavonoids, tannins, phenolics, anthocyanin, ascorbic acid, saponins, alkaloids, and terpenoids. Several studies demonstrated the qualitative presence of alkaloids in methanolic, ethanolic, acetone, ethyl acetate and hexane fractions of Rudraksha bead extract [8-11]. Similarly, terpenoids presence was also reported in methanolic, ethanolic, ethyl acetate and hexane fractions of Rudraksha bead extract (Hardainiyan *et al.,* 2015; Dalei and Sahoo, 2016; Das *et al.,* 2017). Discrepancies were seen in the presence of flavonoid in methanolic and acetone extracts with few studies reporting its presence while other studies showing its absence (Dalei and Sahoo, 2016; Tripathi *et al.,* 2015; Das *et al.,* 2017). Studies conducted by Hardainiyan *et al*., (2015) and Tripathi *et al*., (2015) documented the presence of flavonoids in ethanolic, chloroform and hexane fractions of Rudraksha extract. While presence of tannins was reported in acetone and hexane extracts, discrepancies was recorded in its outcomes for methanolic extracts (Dalei and Sahoo, 2016; Tripathi *et al.,* 2015; Das *et al.,* 2017). Similarly, while saponins were reported to be present in hexane and ethanolic extract, controversies exist for its presence in acetone fractions Presence of phenolic compounds was shown in methanolic, ethanolic, ethyl acetate, chloroform and hexane fractions of Rudraksha bead extract, however controversial results were reported in case of acetone extracts (Jawla and Rai, 2016; Dalei and Sahoo, 2016; Tripathi *et al.,* 2015). Further, none of the previous studies reported the presence of anthocyanins and ascorbic acid in Rudraksha beads both qualitatively or quantitatively.

The current study observed no major changes in the qualitative presence of functional groups in selected Mukhi Rudraksha. Results of FTIR spectroscopy demonstrated the presence of alcohol, phenol, alkanes, aryl ketone, aromatic, alkane, acid, and ether among different Mukhi of Rudraksha. This is in corroboration with the previous study conducted by Tripathy *et al.,* (2016) who documented the presence of multiple functional groups in methanolic extract of five Mukhi Rudraksha bead (Tripathi *et al.,* 2016). The author reported phenol at 3362.94 nm, alkanes at 2943.04 nm, 2831.62 nm, 1452.28 nm, 1375.96 nm, and secondary alcohol at 1105.04 nm synonymous to our study. However, the authors also reported presence of additional functional groups namely carboxylic acid at 1025.63 nm, alkanes at 1452.28 nm and 1375.96 nm, aldehyde at 1726.26 nm, aromatic amines at 1247.62 nm, aromatics at 752.97 nm, halogen compound at 664.68 nm, and halogen derived compound at 608.68 nm which were not seen in our study (Tripathi *et al.,* 2016). Further, the present study also documented the presence of additional group's viz. carbonyls, aryl ketones, aromatic compounds, and nitrogen compounds in all three types of Rudraksha. The differences in the functional group outcomes with the previous study is attributed to the use of solid Rudraksha powder in current study as opposed to methanolic extract in previous study. The outcomes of functional group analysis further support the findings of quantitative phytochemical analysis. FTIR spectra profile at the wave number range of 3200-3600 cm^{-1} and 1720-1740 cm^{-1} demonstrated the –OH group and the C=O carbonyl group of flavonoids respectively (Triyasmono *et al*., 2020). The stretching vibration of –OH group in the wave number range of 3200-3600 cm^{-1} together with the strong peak at 2850-3000 cm^{-1} for CH groups represent the phenolics presence in all Rudraksha (Kesur *et al.,* 2016). A strong absorption in the range of 3200-3600 cm^{-1}, 2850-3000 cm^{-1}, 1720-1740 cm^{-1}, and 1680-1700 cm^{-1} due to hydroxyl groups, alkane group, carbonyl group, and acid group respectively represent the tannins (Wahyono *et al.,* 2019). Anthocyanins can be demonstrated through the character-

istic's peaks of C-H group in the range of 2850-3000cm^{-1} (Favaro *et al*., 2018). Characteristic peaks of hydroxyl group together with C=O and C=C stretch at wave number range of 1680-1700 cm^{-1} and 1400-1600 cm^{-1} indicated the presence of ascorbic acid (Lohmann *et al.,* 1984). Strong and broad stretch of O-H, C=O, C-H, and C=C at wave number range of 3200-3600 cm^{-1}, 1720-1740 cm^{-1}, 2850-3000 cm^{-1} and 1400-1600 cm^{-1} indicate saponins (Almutairi and Ali, 2015). FTIR absorption spectra at 2850-3000 cm^{-1}, 1720-1740 cm^{-1}, 1400-1600 cm^{-1}, 1080-1360 cm^{-1}, 2210-2260 cm^{-1}, and 1000-1150 cm^{-1} for C-H, C=O, C=C, C-N, N-H, and C-O-C indicate the characteristic alkaloids (Fachriyah *et al.,* 2018). FTIR spectra of C-H stretch (2850-3000 cm -^{1}), C=O stretch (1720-1740 cm^{-1}), broad O-H stretch (2850-3000 cm^{-1}), and C-O stretch (1000-1150 cm^{-1}) represent the terpenoids groups (Boughendjioua and Djeddi, 2017). The UV-Vis spectrum of selected Mukhi Rudraksha demonstrated the absorption profile in the band UV spectrum only obtained at wavelengths of 256nm, 287.5 nm, 311.5 nm, and 335.5 nm. Tripathy *et al.* (2016) documented the presence of phenolic and flavonoid compounds at 318.00nm and 245.00 nm respectively in methanolic extract of five Mukhi Rudraksha beads [12]. Therefore, it is implicated that the derived wavelengths represent the presence of phenolic and flavonoid derivatives in 3MR, 4MR, and 5MR.

The elemental analysis of different Mukhi Rudraksha revealed lowest carbon, hydrogen, nitrogen, and C/N ratio in 3MR whereas the same Mukhi Rudraksha possess maximum oxygen content. The results of this study cannot be compared as no previous findings were documented in literature.

Overall, the present study outcomes provided the quantitative evidences with noticeable variations in the physical, physicochemical, phytochemical, and elemental characteristics of 3MR, 4MR, and 5MR. In traditional system of medicine, Mukhi wise specific pharmacological actions have been documented. For instance, while 3MR has been quoted to be specifically beneficial in menstrual disorders, 4MR and 5MR were reported to be effective against nervous system disorders and metabolic syndrome respectively. While the current study found significant variations in the compositional characteristics of 3MR, 4MR, and 5MR, its connection with the differences in the pharmacological activity cannot be commented upon due to two reasons. First, this study found the presence of all components in each Mukhi Rudraksha and second, the preliminary nature of the research. To confirm the traditional concepts related to the differences in Mukhi wise pharmacological action, future studies are warranted for the identification of active biomarkers in different Mukhi Rudraksha. The present study also observed that 5MR possessed the maximum concentration of major phyto constituents. Therefore, it is hypothesized that 5MR may exert better therapeutic action when administered systemically; however future research is warranted to ascertain the same.

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