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Diksha Uniyal
M.V.Sc Scholar, Department of Animal Nutrition, College of Veterinary and Animal Sciences GBPUAT, Pantnagar, Uttarakhand, India

Anshu Rahal
Associate Professor, Department of Animal Nutrition, College of Veterinary and Animal Sciences GBPUAT, Pantnagar, Uttarakhand, India

Corresponding Author
Diksha Uniyal
M.V.Sc Scholar, Department of Animal Nutrition, College of Veterinary and Animal Sciences GBPUAT, Pantnagar, Uttarakhand, India

Phytochemical and proximate analysis of mango leaves and yellow mustard seed

Diksha Uniyal and Anshu Rahal

Abstract
This study was conducted to evaluate chemical composition and phytochemicals present in mango leaf and yellow mustard seed. Yellow mustard seed and mango leaves were collected for estimation of proximate analysis and qualitative presence of phytochemicals. Results indicated that mustard seed contains higher content of crude protein, ether extract and glucosinolate content compared to mango leaf while total phenol content was higher in mango leaf. As both yellow mustard seed and mango leaves were found nutrient-rich they can be used in animal feeding if antinutritional content is reduced.

Keywords: Chemical composition, mango leaf, yellow mustard seed, phytochemicals

Introduction
Mustard has been used as spice and both food and medicine in *Ayurveda*. Mustard seed are small round in shape, usually 1-2 mm in diameter and varying in colour from yellowish white to black**.** Mustard is the third leading source of vegetable oil in the world after soybean oil and palm oil. Mustard is grown as major oil seed crop in most parts of the world. The utilization and marketing of these mustard meals in livestock is very limited due to the presence of anti-nutritional factors in form of mainly 'glucosinolates', which are a group of sulphur containing secondary plant metabolites produced mainly in Brassicaceae family. Glucosinolates themselves are not toxic however their hydrolytic products are toxic. Mustard seed cleanse the cranial cavity, are used for decoction enema, have anti-prurient activity and induce emesis. Mango tree (*Mangifera indica* L.) is known worldwide and is a tropical plant belonging to Anacardiaceae family, mostly cultivated as fruit tree. In traditional Chinese medicine, mango leaves were used for treatment of diabetes and asthma. According to Ayurveda, varied medicinal properties are attributed to different parts (bark, roots, and leaves) of the mango tree. But still knowledge is lacking about glucosinolate content and phytochemicals presence in yellow mustard seed and mango leaf so that they can be included in animal diet.

Materials and Methods
In this experiment, required quantities of yellow mustard seed was procured from the local market Pantnagar (Uttarakhand), India. Mango leaves were harvested from the Department of Horticulture, G.B.P.U.AT, Pantnagar. Taxonomic classification of mango and yellow mustard seed is given in Table1.The leaves were air dried overnight under the shade in order to avoid bleaching of the green colour, until they became crispy. Then they were dried in hot air oven at 60-70ºC and then finely grounded to powder by an electric feed grinder and stored in closed and dry container. Dried yellow mustard seed were ground to powder using mixer grinder and refrigerated in air tight containers until used. Proximate composition of mango leaves and mustard seed was done to assess their nutritional value using AOAC (2000) [3].

Table 1: Taxonomic classification of mango and yellow mustard seed

Taxonomy	Mango	Yellow Mustard
Kingdom	Plantae	Plantae
Class	Magnoliopsida	Magnoliopsida
Subslass	Rosidae	Dilleniidae
Order	Sapindales	Capparales
Family	Anacardiaceae	Brassicaceae
Genus	*Mangifera*	*Brassica*
Species	*indica*	*rapa*
Cultivar/variety	*Dasheri*	*Trilocularis*
Scientific name	*Mangifera indica* L. cv. *Dasheri*	*Brassica rapa*L. var. *Trilocularis* (Roxb.)

Calcium Estimation

Calcium was estimated in the acid mineral extract prepared from ashing of sample and then dissolving in dilute hydrochloric acid extraction. An aliquot of 10 ml mineral extract (dilute to 100 ml with distilled water) was transferred into a 500 ml beaker. Then, two drops of methyl red indicator were added. Diluted ammonia solution (50%) was added dropwise to develop a faint yellow colour which was again adjusted by adding dilute HCL drop wise with constant stirring so as to get faint pink colour. It was diluted to 150 ml and bring to boil. About 10 ml saturated ammonium oxalate solution was added with constant stirring. If the pink colour of the solution changes to orange or yellow, add HCL drop wise until the colour again changes to pink. The contents were heated on a hot plate for about 5 minutes and kept overnight for proper precipitation of calcium oxalate. On the next day, contents were filtered through Whatman filter paper no. 40 with minimum five washings with dilute ammonium hydroxide solution (2%) on the filter paper. After that, filter papers containing whitish calcium oxalate precipitate was quantitatively transferred into a beaker (500ml) and dissolved by adding mixture of 125ml of water and 5 ml of concentrated H_2SO_4. Heat the contents of beaker to 70ºC.The solution is titrated against standard N/10 potassium permanganate (KMnO4) solution. A light pink colour which last for 15sec is considered as an end point. Calcium (%) in the sample was calculated as follows:

$$\text{Ca (\%)} = \frac{(V1 - V2)\ (ml) \times 2.004 \times \text{dilution factor} \times 100}{\text{Wt of the sample} \times 1000}$$

Where,
V1=initial reading of the burette (ml)
V2=final reading of the burette (ml)

Phosphorus estimation

50 ml aliquot of acid soluble mineral extract was taken in a beaker. 20 ml freshly prepared ammonium molybdate working solution was added and stirred without heating. Precipitate was allowed to stand overnight, filtered through Whatman filter paper no.42 and then washed with 3% potassium nitrate solution to remove acidity. Precipitate was transferred with filter paper back to the beaker and dissolved in sufficient quantity of NaOH solution (0.1N) with help of burette. About 4-5 ml of excess NaOH solution was added. Total volume of the 0.1 N NaOH solution used was recorded. Few drops of phenolphthalein indicator solution were added and excess of alkali was titrated with 0.1N standard nitric acid solution.

$$\text{Phosphorus\%} = \frac{V1 - V2 \times 0.1347 \times \text{dilution factor} \times 100}{W \times 1000}$$

Where,
V1=Volume of 0.1 N standard NaOH solution used (V_1)
V2=Volume of 0.1N standard nitric acid used (V_2)

Glucosinolate estimation

Spectrophotometric estimation (Mawlong*et al.*, 2017) [22] was done using methanolic extract prepared by homogenizing 0.1 g defatted sample in a 2 ml vial with 80% methanol. Total glucosinolate was calculated by putting the OD of each sample taken at 425 nm into the predicted formula y = 1.40 + 118.86 × A_{425}, where A is absorbance at 425 nm.

Total phenols estimation

The total phenolic content was estimated by Folin-Ciocalteu method as described by Singleton *et al*. (1999) [34] with slight modifications. Aqueous extract of mango leaves and mustard seed powder was used for total phenol estimation. To 0.1 ml extract (1mg/ml), 0.5 ml of distilled water was added followed by 50µl of Folin-Ciocalteau reagent (FCR). After 3-5 minutes, 0.5 ml of 20% Na_2CO_3 was added and the final volume was raised to 2 ml with the help of distilled water. The mixture was then allowed to stand for 90 minutes at room temperature and the absorbance of the resulting blue coloured mixture was recorded at 765 nm against blank. The absorbance of the standard (gallic acid) was plotted and the total phenolic contents of the extracts were expressed in terms of gallic acid (GA) equivalents from the formula:

$$C = (c \times V)/m;$$

Where,
C = Total content of phenolic compounds, µg/mg or mg/gm extract, in GA equivalents,
C = The concentration of gallic acid established from the calibration curve (µg/mL),
V = The volume of extract (mL), and m = the weight of crude plant extract (mg).

Qualitative Phytochemical Analysis of Aqueous Extract of Mango Leaves Powder and Mustard Seed Powder

1. **Phenolic compounds (Ferric chloride test):** 50 mg aqueous extract were dissolved in 5 ml of distilled water. To this few drops of 5.0% neutral ferric chloride solution was added and the development of dark green colour indicated the presence of phenolic compounds
2. **Flavonoids:** 200 mg of the aqueous extract were dissolved in 2ml methanol and heated. Few turnings of magnesium meat were added to the mixture followed by the addition of a few drops of concentrated hydrochloric acid. The appearance of an orange to red colouration was considered as indicative of the presence of flavonoids.
3. **Saponins:**50 mg extracts were diluted in 20 ml distilled water. The solution were then shaken in graduated cylinder for 15 minutes to see a layer of foam up to 2 cm which indicated saponins.
4. **Tannins:** 50 mg of extract was boiled in 20 ml of distilled water. A brownish green or blue-black colouration resulting upon the addition of 0.1% ferric chloride solution indicated the presence of tannins in the extract.
5. **Terpenoids:** About 0.5g of the extracts were dissolved in 3ml of chloroform and filtered. Concentrated sulphuric acid was slowly added to the filtrate to form a lower layer. A reddish brown colour at the interface indicated the presence of terpenoids.
6. **Glycosides-Borntrage's test:** 50 mg of aqueous extract were hydrolyzed with 5 ml concentrated HCL for 2 hours in water bath and filtered. 2 ml of filtered hydrolysates were taken in a test tube and 3ml of chloroform was added. The chloroform layer was separated by shaking and 10% ammonia solution was added to this to see the appearance of pink colour which indicates the presence of glycosides.
7. **Carbohydrates and sugars -Molish's test:** 100 mg of dried extracts were weighed and dissolved in 5 ml distilled water and subjected to the following tests. 2 ml

of filtrates were taken in a test tube and two drops of alcoholic solution of α-napthol was added. The solutions were mixed properly and 1 ml concentrated H_2SO_4. was added slowly along the sides of the test tubes. A violet ring indicated the presence of carbohydrates.

8. **Fixed oils and fats -Spot test:** The presence of fixed oil was described by pressing small quantity of extract between fingers and the presence of oil stain indicated the presence of fixed oil
9. **Alkaloids-Mayer's test:** For detection of alkaloids, 1.0 g of dried extract was dissolved in 10 ml of 1% HCl by stirring under steam bath. The solution was then filtered and the filtrate was tested with Mayer's reagent. 2 ml of filtrate were taken in a test tube and one or two drops of Mayer's reagent was added gently to the test tube. A white creamy precipitate indicates presence of alkaloid.

Mayer's reagent: 1.36 g $HgCl_2$ dissolved in 60 ml water and 5.0 g potassium iodide dissolved in 10 ml water was mixed and total made up to 100 ml with water.

Results and Discussion

The results of proximate analysis are given in Table 2 and of different phytochemicals in Table 3.Results indicated that mustard seed contains higher crude protein (21.77%), ether extract (47.99%), glucosinolates (151.28 µmole/gram) compared to mango leaves while higher values were noted for crude fibre (19.5%), Ash (12.00), calcium (2.13%), phosphorus (0.123% and total phenol (87.1 mgGAE/g) in mango leaves. Since mustard seed is an oilseed crop higher fat content was present compared to mango leaf. Aletor and Adegoke (2018) [2] has also reported 23.11% crude protein content in yellow mustard seeds. The results of mango leaves crude protein are comparable with the values (CP 10-28%) in fodder trees reported by Singh (1982) [33]. Aletor and Adegoke (2018) [2] reported 51.60 % ether extract content in yellow mustard seeds. Abel *et al. (*2018) [1] reported 22.40 % crude fibre content in mango leaf. Aletor and Adegoke (2018) [2] reported 9.34 % crude fibre content in yellow mustard seeds. Abel *et al. (*2018) [1] reported 11.25 % ash content in mango leaf and Aletor and Adegoke (2018) [2] reported 3.22 % ash content in yellow mustard seeds. Gazwi and Mahmoud (2019) [11] has reported total phenolic content of aqueous extract of *M. indica leaves* to be 86.20 µg GAE/mg extract. Mustard protein has been reported to contain a good balance of the essential amino acids (Goering *et al*. 1960 [13]; Miller *et al.* 1962 [23]; Zeb, 2002 [41]; Thanaseelaan, 2013 [38]). The amino acid composition of the protein of 41 species of Cruciferae stated that the cruciferous seeds were as high in their sulfur-containing amino acids as was commercially available, solvent extracted, soybean meal (Miller *et al.*, 1962) [23].Goering *et al.* (1960) [13] described mustard as an unusual protein because of its high content of methionine and tryptophan.The pungent properties of mustard act as a preservative against the action of yeasts and moulds and are thought to be more effective in this respect than sulphur and benzoic acid (Corran and Edgar, 1933) [7]. Mustard has also been used in the field of medicine. Long term consumption of mustard oil produces a number of beneficial effects such as prevention of dyslipidemia, coronary artery diseases, atherosclerosis and colon cancer (Singh *et al*.,1997) **[31].**Mustard seeds have shown hypoglycemic effect in rats (Malik *et al.*, 2011 [20]; Khan *et al*., 1995 [18] and Srinivasan, 2005 [36]). Mustard oil is effective in cardiovascular diseases (Singh *et al.*, 1997 [31]; Rastogi *et al.*, 2004 [29]) and also used in management of abdominal swelling, skin diseases, epilepsy, insanity and frozen thigh. It is considered to be a lipid lowering agent, anthelminitic and used in diseases affecting the head, hemorrhoids and wounds. Internally, mustard oil is used to season food and recommended in elephantiasis and retention of placenta. It is also used for urethral infiltration. In filariasis, mustard oil is recommended for internal use with the juice of the leaves of *Pongamiaglabra* (Manohar *et al*., 2009) [11].

The qualitative phytochemical analysis of the aqueous mango leaves extract revealed the presence of phenols, flavonoids, saponins, tannins, terpenoid, glycosides, carbohydrate & sugars, fixed oils and fats and alkaloids. In case of aqueous yellow mustard seed extract phenols, flavonoids, tannins, terpenoids and fixed oils and fats were found to be present while saponins, glycosides, carbohydrate and sugars and alkaloids were found absent. Earlier reports have also indicated that mango leaves are rich in polyphenols (Sferrazzo*et al*., 2019) [30]. Gazwi and Mahmoud (2019) [11] reported the presence of phytochemical constituents as flavonoids, phenol, steroids, glycosides, saponins and terpenoids in *M. Indica* aqueous extract. Phytochemical research from different parts of mango plant has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols. It is used medicinally to treat ailments such as asthma, cough, diarrhoea, dysentery, leucorrhoea, jaundice, pains, and malaria. Aqueous extract of mango leaves has shown significant analgesic activity in rats (Mohanvelu *et al.*, 2015) [25]. Its leaves contain phenolic constituents such as caffeic acid (Singh *et al.*, 2004) [32] polyphenols such as mangiferin and gallic acid (Barreto *et al*., 2008) [4], flavonoids (Kanwal *et al.*, 2010) [17], volatile compounds (Gebara *et al*., 2011) [12]. Mango leaves is a good source of mineral elements (Jhaumeer *et al*., 2018) [16]. The natural C glucoside, xanthonemangiferin has been reported in leaves of *Mangifera Indica*. Mangiferin includes antioxidant (Leiro *et al*., 2003 [19]; Stoilova *et al.*, 2005) [37], radioprotective (Jagetia and Venkatesha, 2005) [15], antitumor (Yoshimi *et al*., 2001) [40], anti-inflammatory (Garrido *et al*., 2004) [10], antidiabetic (Dinesh kumar *et al.*, 2010 [8]; Muruganandan *et al.*, 2005 [27]) and lipolytic (Yoshikawa *et al.*, 2002) [39] property. Mango leaves extract have also shown properties like antimicrobial, anthelminthic, antiallergic (Hannan *et al*., 2013 [14]; Zhang *et al*., 2014 [42]). Phenolic compounds such as flavonoids have antioxidant activity (Skerget *et al*., 2005 [35]; Bhatia *et al*., 2011 [5]; Fernandez-Ponce *et al*., 2015 [9]), which is higher than that of β-carotene (Pereira and Meireles, 2007) [28]. Mango leaves extract can be used as natural preservative in food (Morsi *et al*., 2010) [26] and as anti-ageing compound in cosmetic products (Charrier *et al.*, 2006) [6]. It also has pharmaceutical applications such as chemoprevention in diseases related with oxidative stress (Mohan *et al.*, 2013) [24].

Table 2: Proximate composition (%) of mango leaf powder and mustard seed powder on dry matter basis

Particulars	Mango leaf powder	Mustard seed powder
Dry matter (%)	48.85	97.11
Crude protein (%)	10.5	21.77
Ether extract (%)	5.98	47.99
Crude fibre (%)	19.5	9.64
Ash (%)	12.00	4.0
Nitrogen free extract (%)	52.02	16.6
Calcium (%)	2.13	0.73
Phosphorus (%)	0.123	0.09
Glucosinolate (µmole/g)	-	151.28
Total phenol (mgGAE/g)	87.1	21.86

Table 3: Qualitative phytochemical analysis of aqueous extract of mango leaves powder and mustard seed powder

Phytochemicals	Method	Observations	
		Mango leaves	Yellow mustard seed
Phenols	Ferric chloride test	+	+
Flavonoids	--	+	+
Saponins	--	+	-
Tannins	--	+	+
Terpenoids	--	+	+
Glycosides	Borntrage's test	+	-
Carbohydrates & sugars	Molish's test	+	-
Fixed oils and fats	Spot test	+	+
Alkaloids	Mayer's test	+	-

Conclusion

Yellow mustard seed and mango leaves both being rich in nutrients and phytochemicals possesses antimicrobial, antidiabetic, anti-hepatic, antioxidant properties. Both mustard seed and mango leaf can be included in animal diet as feed additive for their health improvement.

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