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Phytochemical and *in- vitro* antioxidant activities of methanolic extract of whole plant of *Cynodon dactylon* and *Brassica rapa* M27 seed

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Abstract
Nowadays, the need to use natural products became of great importance, as because resistance of micro-organisms to antibiotics is increase day by day and also to decrease the side effects on human beings. A wide range of medicinal plant parts are used to treat numbers of diseases from ancient time. Traditionally *Cynodon dactylon* is used in skin trouble, treatment of wounds, as laxative, brain and heart tonic, emetic, expectorant, carminative, and for pains, inflammations and toothache etc. *Brassica rapa M27 seed* is used as rubefacient and a very good folk remedy for cancer. This work was done to investigate the phytochemical properties and the antioxidant activity of methanolic extracts of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27.* In this study free radical scavenging activity was determined by *in-vitro* assay models such as DPPH free radical, reducing ability. Quercetin was used as reference standard. The findings indicated that it contains different phytochemical constituents and promising antioxidant activity of crude extracts of the above mentioned plants in a dose dependent manner and needs further exploration for their effective use in both modern and traditional system of medicine which can be used to treat different types of diseases.

Keywords: Whole plant and seed, *Cynodon dactylon*, *Brassica rapa M27*, antioxidant activity, DPPH

1. Introduction
The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India. Bacteria, in general, possess the genetic ability to acquire and transmit resistance to therapeutic agent. Not today, but from the ancient days itself, our ancestors depended on the plants for cure and medication. Medicinal plants represent a rich source of antimicrobial agents. According to World health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care needs. Now a days most of the synthetic antibiotics are resistance to several bacteria. So there is a need to sift our study towards Plants. The major merits of herbal medicine are low incidence of serious adverse effects and cost 1.
Cynodon dactylon (family-Graminae/Poaceae) has some common name as Bermuda grass, dhoob. *Brassica rapa* M27 (family- Brassicaceae) has some common name oil seed, turnip, rapeseed mustrd oil.

Methods and materials
2.1 Plant material
a. Collection and authentication of sample
Cynodon dactylon plant specimens were collected from Nalbari District of Assam and Seed of *Brassica rapa (Toria-variety M27)* specimens were collected from Regional Agricultural Research Station (Krishi Vigyan Kendra), Shillongani, Nagaon, Assam. The *Cynodon dactylon* plant and Seed of *Brassica rapa (Toria-variety M27)* was collected in the month of February, 2016. The *Cynodon dactylon* plant was kindly authenticated with the standard Herbarium specimen in Botany Department of Guwahati University, Assam(Acct.no:18125 on dated 29th March 2016) and breeder Seed of *Brassica rapa (Toria-variety M27)* was kindly authenticated in Regional Agricultural Research Station (Krishi Vigyan Kendra), Shillongani, Nagaon, Assam (No:2091 on dated 10th march 2016).

b. Preparation of extract 3
Powdered (200 g) were extracted with methanol using Soxhlet apparatus. The methanol was added up to 2 siphons that is up to 500ml. The temperature was set to 700C.The extract was concentrated at 700C to dryness under reduced pressure to yield a dried crude methanol extract. Similarly, the extracts were then stored at 4 ºC till the time of use. The% yield of methanolic extract of *Cynodon dactylon and Brassica rapa M27* was found to be 12.6% w/w and 11.5% w/w respectively.

2.2 Preliminary phytochemical Test 3,4,5
2.2.1 Test for Alkaloids: Solvent free extract, 50mg was stirred with few ml of dilute hydrochloric acid & filtered. The filtrate was tested carefully with various alkaloidal reagents as follows:

a. Mayer's test: To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates test as positive.

b. Dragendorff's test: To a few ml of filtrate, 1 or 2ml of Dragendorff's reagent was added by the side of the test tube. A prominent yellow precipitate indicates test as positive.

2.2.2 Test for Carbohydrates: The extract (100mg) was dissolved in 5ml of water & filtered. The filtrate was subjected to the following test-

a. Molish's test: To 2 ml of filtrate, 2 drops of alcoholic solution of alpha-naphthol was added, the mixture was shaken well & 1ml of conc. H2SO4 was added slowly along the side of the test tube & allowed to stand. A violet ring indicates the presence of carbohydrate.

b. Barfoed's test: To 1 ml of filtrate, 1 ml of Barfoed's reagent was added & heated on a water bath for 2 min. Red ppt. indicates presence of sugar.

2.2.3 Test for Glycoside
50gm of extract was hydrolysed with concentrated hydrochloric acid for 2hr on a water bath, filtered & the hydrolysate was subjected to the following test-

a. Borntrager's test: To 2ml of filtered hydrolysate, 3ml of CHCl3 was added & shaken, CHCl3 layer was separated & 10% NH3 solution was added to it; pink color indicates the presence of glycosides.

b. Keller Killiani Test: Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

2.2.4 Test for Tannins
a. Ferric chloride test: About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% FeCl3 were added. brownish colour indicates the presence of Tannins.

b. Gelatin Test: The test solution was treated with 1% Gelatin solution containing 10% NaCl, a white precipitate indicates the presence of Tannin.

2.2.5 Test for flavonoids
a. Lead acetate test: To the alcoholic solution of the extract few drops of lead acetate solution (10%) was added, appearance of yellow precipitate indicates the presence of flavonoids.

b. Ferric chloride test: Alcoholic solution of the extract and a few drops of ferric chloride was added, appearance of green colour indicates the presence of flavonoids.

2.2.6 Test for Saponin glycosides
Foam test – 5ml of the extract was diluted with 10ml of distilled water; the solutions were then vigorously shaken and observed on standing to obtain persistent of foam.

2.3 *In-vitro* antioxidant activity 6-21
An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, flavonoids scavenge free radicals such as peroxide, hydroperoxide inhibit the oxidative mechanism. Herbal plants considered as good antioxidant since ancient times. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. In this Experiment free radical scavenging activity of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27* were determined by different *in-vitro* assay methods such as DPPH free radical, reducing ability. Quercetin was used as reference standard.

2.3.1 DPPH radical scavenging activity Principle
A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPH-H and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form, results in decolourization (yellow colour) with respect to the number of electrons captured. More the decolourization more is the reducing ability. Radical scavenging activity increased with increasing percentage of the free radical inhibition.

Procedure
Free radical scavenging activity of different extracts of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27* were measured by (DPPH). In brief, 0.1 mM solution of DPPH in methanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in methanol at different concentration (20, 40, 60, 80, 100 µg/ml). Here, only those extracts are used which are solubilise in methanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room

temperature for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu).6 Reference standard compound being used was Quercetin and experiment was done in triplicate. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation; Percentage inhibition = (1– absorbance of test/absorbance of control) × 100.

2.3.2 Reducing power assay Principle

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, ferrous chloride are found as a product. Which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples.

Procedure

In this case 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v) were added to 1.0 ml of different concentrations (200 to 2000 µg/ml) of the extract fractions .The mixture was shaken thoroughly and incubated at 50 °C for 20 min at room temperature followed by the addition of 2.5 mL of Trichloro acetic acid (10% w/v) to it and centrifuged at 3000 rpm for 10 min to collect the upper supernatant layer of the solution. Now 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%) were added to it .Finally the absorbance of mixture was tested spectrophotometrically at 700 nm using an appropriate blank of 3.5 ml of potassium ferrocyanide solution sample.

3. Result and Discussion

The investigation details is mentioned below

Table 1: Phytochemical Screening result of *Cynodon dactylon* methanolic extracts

Test	Methanol
Alkaloids	+
Carbohydrates	+
Glycoside	-
Flavanoids	+
Tannins	+
Saponins	+

"(+)" means present and **"(-)"** means absent

Table 2: Phytochemical Screening result of *Brassica rapa M27* methanolic extracts

Test	Methanol
Alkaloids	+
Carbohydrates	-
Glycoside	-
Flavanoids	+
Tannins	+
Saponins	+

"(+)" means present and **"(-)"** means absent

Table 1 shows the results of Phytochemical analysis of the methanolic extract of *Cynodon dactylon* of the family *Poaceae* showed that all critically important secondary metabolites were present in the methanolic extract.

Table 2 shows the results of Phytochemical analysis of the methanolic extract of *Brassica rapa M27* of family *Brassicaceae* showed that all critically important secondary metabolites were present in the methanolic extract.

Table 3: Reducing ability of Methanolic Extract of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27* With respect to standard Quercetin at 700 nm

Sl. No	Concentration (µg/ml)	Absorbance at 700 nm		
		Whole plant of *Cynodon dactylon*	Seed of *Brassica Rapa M27*	Quercetin
1	200	0.352	0.254	2.041
2	400	0.705	0.602	2.521
3	600	1.050	0.932	2.736
4	800	1.402	1.423	2.814
5	1000	1.73	1.871	2.896
6	2000	2.82	2.617	2.974

Table 4: DPPH Radical Scavenging Activity of Methanolic Extract of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27*

Sl. No	Concentration (µg/ml)	Absorbance at 517 nm		
		Whole plant of *Cynodon dactylon*	Seed of *Brassica Rapa M27*	Quercetin
1	20	1.761	1.662	2.889
2	40	1.423	1.324	2.542
3	60	1.021	0.876	1.924
4	80	0.872	0.753	1.821
5	100	0.467	0.439	1.252

Table 5: Percentage (%) inhibition of Methanolic Extract of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27.*

Sl. No	Concentration (µg/ml)	Percentage (%) inhibition of Methanolic Extract	
		Whole plant of *Cynodon dactylon*	Seed of *Brassica Rapa M27*
1	20	39.1	42.5
2	40	44.1	48.0
3	60	47.0	54.5
4	80	52.2	58.7
5	100	62.7	64.9

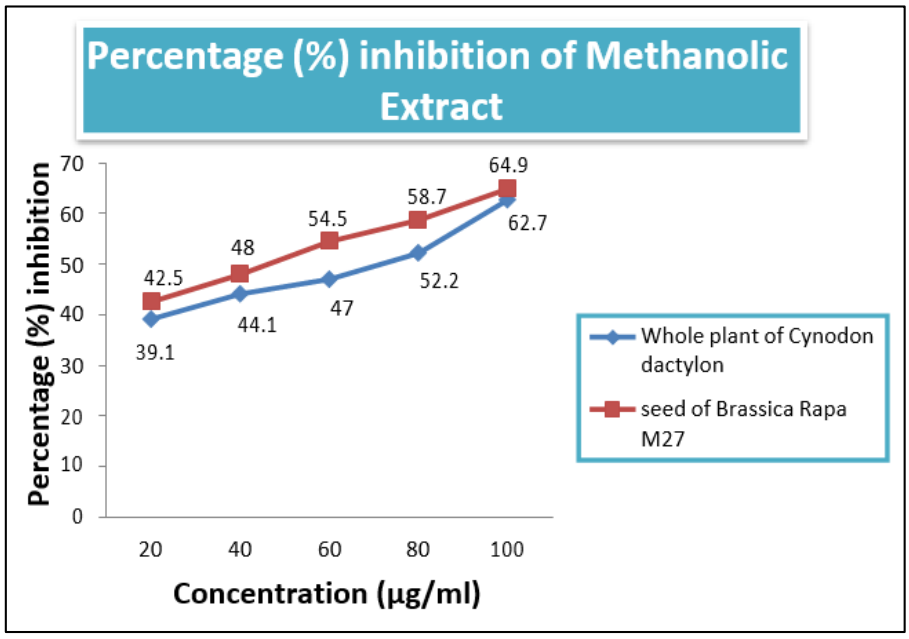

Fig 1: Percentage (%) inhibition of methanolic extract of whole plant of *Cynodon dactylon* and seed of *Brassica rapa M27.*

In the present study, we have investigated the phytochemical constituents and *in-vitro* Antioxidant activity of Methanolic extract of whole plant of *Cynodon dactylon* and *Brassica rapa M27* seed. In the phytochemical investigation, we have found that, the Methanolic extract of *Cynodon dactylon* contains carbohydrates, alkaloids, flavonoids, tannins, saponin whish is listed in Table 1 and *Brassica rapa M27* contains alkaloids, flavonoids, tannins, saponin as constituents which is listed in Table 2.The study of antioxidant activity by using two models ystems i.e. DPPH method and reductive ability which is shown in tables.

In Table 3 shows the Reducing ability With respect to standard Quercetin at 700 nm.

In Table 4 shows the DPPH Radical Scavenging Activity and Table 5 shows the Percentage (%) inhibition of Methanolic Extract of whole plant of *Cynodon dactylon* and seed of *Brassica rapa* M27. It shows that increase in the concentration of the extracts increase the percentage (%) of inhibition which is graphically represent in Fig 1.

4. Conclusion

By this we can conclude that the methanolic extract of whole plant of *Cynodon dactylon* (Family-poacea) and *Brassica rapa* M27 seed (Family-Brasicacea) had shown the presence of different type of phytochemical constituents. From the result of antioxidant activity it can be concluded that the Methanolic extracts shows *in-vitro* antioxidant activity in Dose dependant manner.

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