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ANTI-OXIDANT ACTIVITY OF *NIRGUNDI* (*Vitex nigundo* Linn) *KALPA* – A COMPARATIVE ANALYTICAL EVALUATION

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ABSTRACT

Craving for longevity is one among distinct instinct of human being since ages. The term *Rasayana* has been used to enhance quality of life by acharyas of Ayurveda. Different forms of *Rasayana* preparations have been added to literature at different intervals of evolution of Ayurveda by different authors. *Rasayana Kalpa*s are said to improve quality of life by protecting cells and organs from damage causing radicals, which is popularly known as Anti-oxidant activity. *Nirgundi Kalpa* is usage of Nirgundi moola churna with variable Anupana for specific outcomes, which is uncommonly, used in general practice. *Nirgundi* (*Vitex negundo* Linn) is also said to possess memory enhancing properties. Hence evaluation of nootropic activity becomes essential, which can be basically done through analytical procedures and further translated to higher studies. Present study is carried out to evaluate DPPH radical scavenging assay of *Nirgundi Kalpa* in comparison with *Nirgundi Moola churna* and Honey. *Nirgundi Kalpa* exhibits considerably good anti-oxidant activity compared to concentration of phenolics present in *Nirgundi moola churna*.

KEYWORDS: Nirgundi Kalpa, DPPH, Phenolics and Flavonoids

INTRODUCTION

Healthy body needs healthy mind and hence Ayurveda emphasized prasannata of Atma and Manas in defining healthy person. *Smrithi, Medha and Vaaksiddhi* are mentioned as outcome of *Rasayana Chikitsa*[1]. *Medha* and *prajna* are mainly related with cognition and cognitive enhancers have attained significance in the wake of increased prevalence of cognitive dysfunction such as dementia, Alzheimer's disease etc. Cognition is defined as a process of acquiring knowledge and understanding through thoughts, experience and senses[2]. It encompasses knowledge, attention, learning, memory, judgment, evaluation, reasoning and problem solving. Human cognition is conscious, unconscious, concrete or abstract as well as intuitive and conceptual Cognitive dysfunction is a condition in which there is a loss of intellectual function such as thinking, remembering and reasoning of sufficient severity to interfere with daily functioning[3]. Herbs, which are presently at use for memory promotion, are already red-listed. Much more are declared as endangered species. In order to have a strong basis for the usage of the drug, the activity of the drug has to be established experimentally.

Cognitive enhancers are said to modulate neuron impulses through neuro-transmitter mechanism either by enhancing or supporting CNS function. CNS functions also depend on the health of neurons as neurons may undergo degeneration due to excessive stress[4]. Hence neuro-protective function of Medhya *Rasayana* needs to be evaluated through suitable parameters. *Nirgundi (Vitex negundo.* Linn*)* is one such abundantly available plant and mentioned as Smritida (memory promoter)[5]. *Nirgundi Kalpa* is group of formulations mentioned by Govinda Das in his text Bhaishajya Ratnavali, *Rasayana*dhikara[6], which is said to possess different actions based on anupana and dosage forms. *Nirgundi moola* is the base material and can thus be used with water or with Madhu[7].

Many studies for neuro protective activities are performed through anti-oxidant activity[8]. Effect of *Kaala parinaama* as well as *Samyoga* with *Madhu* has to be analyzed as *Nirgundi* kapla is prepared using *Madhu*. *Madhu* being known for its yogavahitwa[9] is emphasized for the changes that can bring in *Nirgundi moola Choorna*. Hence the present study is intended to execute the phyto-pharmaceutical studies of *Nirgundi Kalpa* with special emphasis to anti-oxidant potential.

MATERIALS AND METHODS

Fresh *Nirgundi* roots were collected from its natural habitat in Mysore, Karnataka. The chemicals that were used are distilled water, sulfuric acid solution, Mayer and Dragendorff's reagents, Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, sodium hydroxide, sodium nitrite, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, ferric chloride, phosphate buffer, Ascorbic acid and Gallic acid were procured from local vendor.

Extraction[10]

Collected *Nirgundi* roots (Image 1) were washed with water, chopped, and dried for 3 days under sunlight. The dried root bark was desiccated and was dried using hot air oven under 60^0 for 5-6 hours, later ground manually in the pulveriser and filtered using cloth to obtain fine powder. 300 grams of powder was mixed with double the quantity of honey (v/v) in an earthen pot (Image 3), lid sealed with mud wrapped cloth and was kept in husk for a span of one month that yielded the *Nirgundi Kalpa*[11]. The dried root powder was kept in plastic cover and stored in refrigerator to avoid contamination for same duration. Both samples were used for extract preparation. Extraction was done using maceration method. The plant material (25g) was macerated with 1:10 times of alcohol (v/v) for 2 hours and was subjected for Soxhlet extraction till complete exhaustion of the samples. Extracts were evaporated on a water bath until dried extract with constant weight was obtained. Extracts were stored in a refrigerator under 4°C until time of use.

Phytochemical screening

Preliminary phytochemical screening of alcoholic extract of NK (*Nirgundi Kalpa*) and NM (*Nirgundi Moola churna*) was performed to test the presence or absence of various primary and secondary metabolites such as carbohydrates, proteins, alkaloids, steroids, terpenoids, phenols, flavonoids, glycosides, etc. using standard methods[12].

Total Phenolic Content

The total phenolic content of the extract was determined by the Folin–Ciocalteu

method[13]. Roughly, 200 µL of crude extract (1 mg/mL) were made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 765 nm. Total phenolic content was calculated from the calibration curve, and the results were expressed as mg/gram of gallic acid equivalent.

Flavonoid content

Flavonoid content of crude extract was determined by the aluminium chloride colorimetric method[14]. 50 µl of crude extract (1 mg/ml) was made up to 1 ml with distilled water, mixed with 4 ml of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg of quercetin equivalent per gram dry weight.

Antioxidant Properties

1,1-Diphenyl-2-picryl-hydrazyl assay

Antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay[15]**,** as described earlier with some modifications. Roughly, 200 µL of each extract (20–200 µg/ml)and standard (2–20 µg/ml) were mixed with 3.8 ml DPPH solution and incubated in the dark at room temperature for 40 minutes. Absorbance of the mixture was then measured at 517 nm. Ascorbic acid was used as a positive control. Ability of the sample to scavenge DPPH radical was determined by following equation.

% DPPH Radical Scavenging = (Acontrol – Asample)/ Acontrol x 100%

Meanwhile, the IC_{50} was computed based on the regression equation y = ax +b.

RESULTS

Nirgundi moola churna (Image 2) was dark grey in color, bitter in taste and having characteristic odor. *Nirgundi Kalpa* (Image 4) was dark brown in color, having sweetish acrid taste and odor of honey (Image 1 and 2). Both extracts were dark brown in color. % extractive values of both *Nirgundi* chrna and *Kalpa* have been mentioned in **Table 1.**

Phytochemical screening.

Based on the results of phytochemical screening, alcoholic extract of NK consists alkaloids, flavonoids, tannins, polyphenols, carbohydrates and proteins. The same phytochemical result was reported for alcoholic extract of NM **(Table 2)**.

Determination of total phenolic contents of NK and NM.

Table 3 shows the total phenolic contents of NK and NM. Total phenolic compounds were reported as Gallic acid equivalents by reference to a standard curve (Graph 1). Total phenolic content was observed more in *Nirgundi moola churna* compared to *Nirgundi Kalpa* and same has been observed with total flavonoids content (Table 4), where quercetin had been taken as reference standard (Graph 2).

ANTIOXIDANT ACTIVITY

1,1-Diphenyl-2-picryl-hydrazyl assay

The DPPH radical scavenging activities of NK and NM are presented in the **(Graph 3 & 4)**. Both the extract showed the concentration dependent increase in the free radical scavenging activity Table 5.

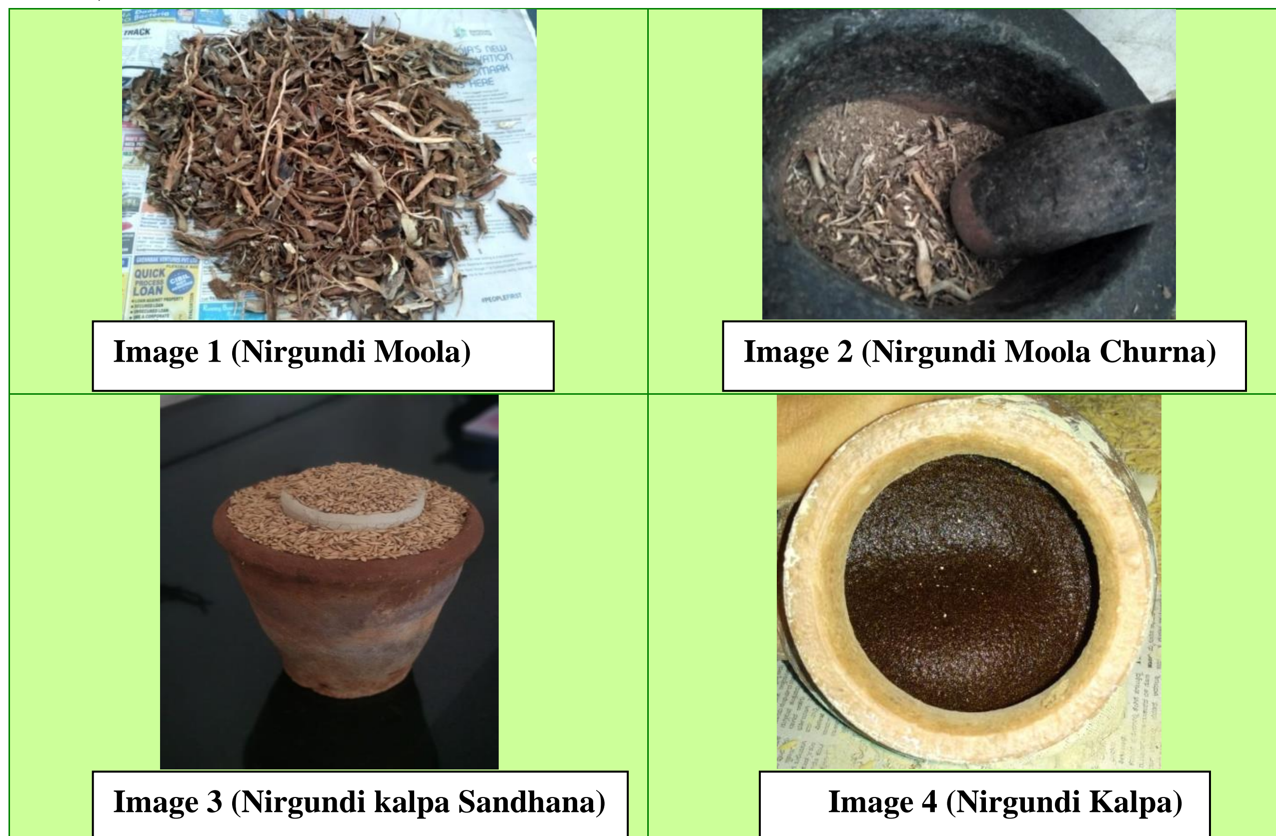


Image 1 (Nirgundi Moola)

Image 2 (Nirgundi Moola Churna)

Image 3 (Nirgundi kalpa Sandhana)

Image 4 (Nirgundi Kalpa)

Table 1: Percentage yield of NK and NM.

Extract	Yield (%)
NK	21.47
NM	28.6

Table 2: Phytochemical screening of NK and NM.

Metabolites	NK	NM
Alkaloids	++	++
Flavonoids	++	+
Tannins	++	++
Saponins	-	-
Glycosides	+	-
Phenolic Compounds	+	+
Phytosterols	+	++
Carbohydrates	+++	+
Protein and Amino Acids	+	-

-Not determined; + Low concentration; ++ Medium concentration; +++ High concentration.

Table 3: Total phenolic content of NK and NM.

Concentration	Phenolic content (mg of gallic acid equivalent / g of dry weight)
NK(1mg/ml)	9.837 ± 0.019
NM (1mg/ml)	20.108 ± 0.057/

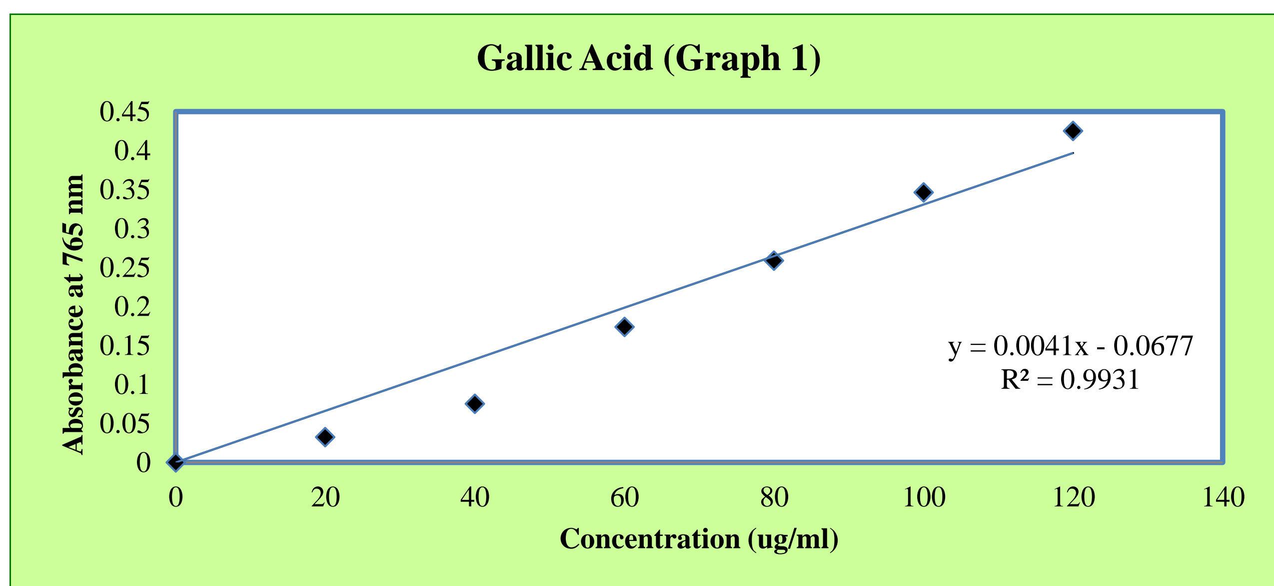
Table 4 : Results of total flavonoid content of NK and NM.

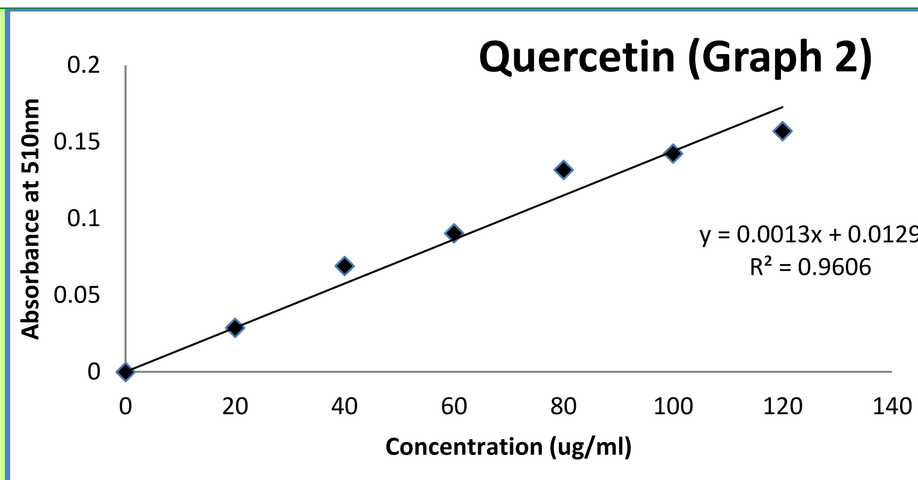
Concentration of extract	Flavonoid content (mg of rutin equivalent / g of dry weight)
NK1mg/ml	1.051 ± 0.02
NM 1mg/ml	3.335 ± 0.052

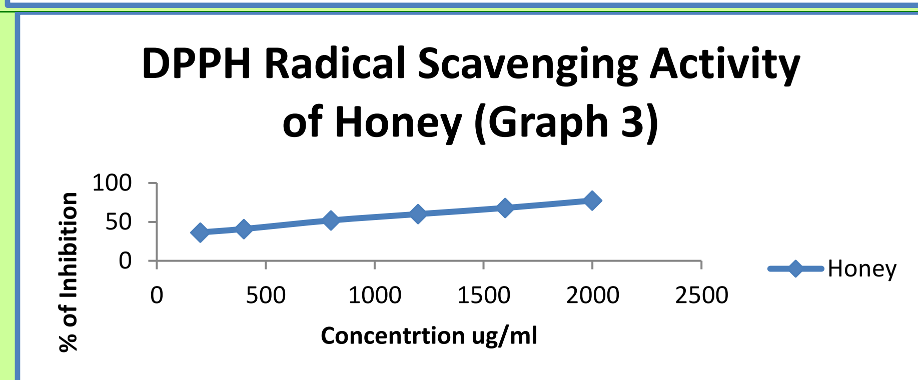
Values are mean ± S.D, n=3

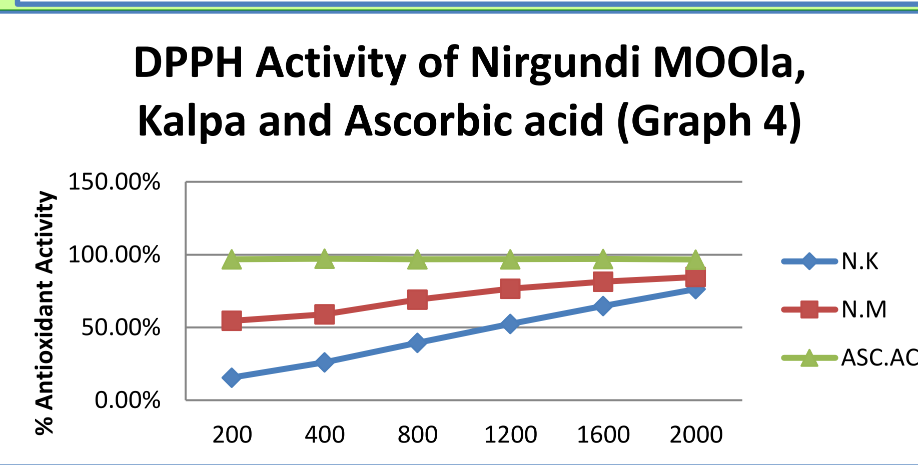
Table 5 : DPPH radical scavenging activity of NK and NM.

Concentration (ug/ml)	DPPH Scavenging %			DPPH Scavenging % Ascorbic acid
	NK	NM	Honey	
200	57.85±6.64	15.57±7.3	31.12±1.5	96.83±1.5
400	62.36±5.53	26.08±6.11	41.37±1	97.14±1.25
800	72.42±3.85	36.15±5.63	50.36±3.2	96.84±1.5
1200	79.89±4.44	52.34±7.13	58.59±2	96.89±1.5
1600	84.86±6.18	64.47±7.09	68.11±1.1	96.93±1.5
2000	87.95±4.51	76.31±5.65	76.57±1.2	96.97±1.5









DISCUSSION

Smrutikara or *medhakara dravyas* are those formulations that possess the capability to improve the Grahana Shakti of an individual[16]. This invariantly can be co-related to the nootropic drugs in current era. As these drugs are co-related in enhancing the cognitive functions in a human, it holds a great importance in current era where every individual wants to excel extraordinarily well in their domain. Though many nootropic preparations exist in present day scenarios like *Rasayana*s, immunity enhancers, studying them scientifically on modern basics is essential for evidence based medical practice.

Many analytical study and evaluations have been carried out on *Nirgundi* as a whole plant and with its different parts[17]. In total Antioxidant activity, DPPH assay is considered here as it is an accurate mechanism to screen the anti oxidant activity by addition of extract in concentration dependent manner of plant extract[18]. These antioxidant capacities are further established by knowing the presence of phenolic contents as antioxidant capacities are related to hydroxyl group of phenolic rings.[19]

There are no studies done with sample of *Nirgundi Moola Churna* with following assays and hence adapted these evaluatory studies. The fraction taken was ethanolic extracts to assess activity of compounds. Phenolics have been reported to possess strong anti oxidant activities and have capacities to donate hydrogen atoms to free radicals. Also from the study, the presence of phenolics support the anti oxidant activity of the sample.

In the sample of *Nirgundi moola* and *Nirgundi Kalpa*, *Kalpa* exhibits a higher Antioxidant potential as per the results. DPPH is a stable organic free radical, which loses its absorption spectrum band at 515 – 528 nm when it accepts an electron or a free radical species. The DPPH assay is a simple, acceptable and most widely used technique to evaluate the radical scavenging potency of plant extracts. The antioxidants are the components of the plants which are capable of enacting the visually noticeable quenching of the stable purple- coloured DPPH radical to the yellow – coloured DPPH.

When compared with the sample extract of *Nirgundi moola Choorna*, the active principles present in Nirgund *Kalpa* will be reduced one half, quantity wise. On analysing the antioxidant activity, *Nirgundi moola Choorna* possess a better activity than the antioxidant activity shown by plain Madhu. Even then in Antioxidant evaluation, when *Nirgundi Kalpa* shows higher potential, the Activity of Madhu on *Nirgundi Kalpa* cannot be ruled out. It can be assumed that the Yogawahi guna[20] of Madhu. Also, on evaluating the Phenolic Content of *Nirgundi Kalpa* and *Nirgundi Moola Churna*, the root powder shows a higher phenolic content. This must be due to the presence Active phytochemicals will be more in quantity compared to the formulation of *Nirgundi Kalpa* as the ratio of *Churna* present in *Nirgundimoola* to *Nirgundi Kalpa* is 2:1 respectively. The result shows that the values are almost double in *Nirgundi Moola* when compared to *Nirgundi Kalpa*.

Graph gradient pertaining to DPPH activity of *Nirgundi Kalpa* was though less in its lower concentrations moved steeply and in higher concentrations, was almost nearing to the point of *Nirgundi churna*. Factor to be noted here is quantitatively presence of *Nirgundi churna* varies in both samples as *Nirgundi Kalpa* had double the quantity of Madhu. Hence better DPPH results of *Nirgundi* kapla must be due to Yogavahitwa activity of Madhu and even due to Kala parinama (processing time) as *Nirgundi Kalpa* was kept for 30 days for maturation.

Hence, as Acharya has mentioned as the usage of the *Nirgundi Kalpa* indicates all its qualities towards a *Rasayana Karma*. This can also be considered as a cognitive enhancer as the Main constituents of *Nirgundi* and Madhu as, they are classically

accepted as Medhya dravyas. Hence the combination of these both can be relied upon and can be implemented for further levels of experiments.

CONCLUSION

Rasayana Karma is group of activities indicated for enhancing quality of life and tissue protection is one among them. Analytical studies though do not establish nootropic activities, but can be considered as corroborative factors determining preliminary activities supporting pharmacological functions. *Nirgundi moola* through phenolic compounds mediate protective role on different organs including brain. *Nirgundi Kalpa*, where honey is one of the ingredients influences anti-oxidant activity to the greater extent, which is evident by the present study. Phytochemical alterations possibly happen during storage phase need to be evaluated through suitable modalities in future studies.

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