Original Article

In Vitro Antioxidant And Anticancer Activity Of Nigella Sativa, Anethum Sowa And Berberis Aristata Herbal Formulation Using MCF-7 Cell Line

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Abstract

Several herbs and plants are well-known for their medicinal and therapeutic properties. We intended to test that the formulation of *Nigella sativa, Anethum sowa* and *Berberis aristata* herbal formulation as aqueous and ethanol extracts have got antioxidant and anticancer activities. Equal quantities of each plant powder were mixed and subjected to aqueous and ethanol solvents for 72 hours by maceration procedure. The extract obtained was used for further studies. Different forms of assays like MTT, ABTS, GCMS and anticancer activities on MCF -7 cell lines were performed. Significant anti-oxidant, free radical scavenging and anticancer activities were noticed in the mixture. The anticancer activity was higher than individual described herbs. We suggest that synchronized action by a mixture of herbs could prove more beneficial in drug concepts than individual drugs.

Keywords: Nigella sativa, Anethum sowa, Berberis aristate, anti-oxidant, anticancer

INTRODUCTION:

Plants, as the primary source of numerous medicines, play an essential and crucial role in both plant and human health. Several herbs and plants are well-known for their medicinal and therapeutic properties. Each plant contains several significant elements that can be employed in the medical industry and can be used to generate various types of medications. are reducers found in ordinary bases such as berries, veggies, and fleshes[1]. Antioxidants are the prevalent natural reducers found in routine foods: vitamin C, vitamin E, vitamin A, and various polyphenols, including flavonoids, anthocyanins, lycopene, and coenzyme Q10 also referred to as Ubiquitin, which is a form of protein. There are numerous sources of antioxidants including plants and herbs. There are innumerable studies that establish the antioxidant and anti-cancer potential of many herbs and their extracts[2-5]. *Nigella sativa* seeds[6] are used in herbal therapy to treat lung disease, hypertension, stomach diseases, liver disorders, immune disorders, tumors, neuro diseases, and other health issues. Antioxidant action, anti-diabetic activity, and anti-inflammatory activity seem to be the most common therapeutic outcomes. The essential oil separated from seeds of *Anethum sowa*[7] has some valuable properties such as anti-diabetic, antispasmodic, inhibiting bacterial growth, reducing inflammation, free radical scavenging, and decreasing blood cholesterol. According to Ayurvedic medicine, *Berberis aristata* [8]helps to cure foe dysentery, wound healing, skin disease, inflammation, diarrhea, jaundice, menorrhagia, and eye diseases. It is used to treat leprosy in the Unani medicinal system, and the plant's root extract is used to treat skin disorders such as ulcers and abrasions. We planned to combine the herbal extracts and study their usefulness in medicine. We aimed to investigate the phytochemical constituents present in the herbal formulations: To estimate the antioxidant activity of the herbal formulations by H_2O_2 assay, FRAP assay, and ABTS assay: And to study the cytotoxicity and anticancer property by performing MTT assay and AO/PI dual staining of herbal formulations using MCF 7 cell line.

METHODS:

Nigella sativa, Anethum sowa and Berberis aristata were purchased from herbal shop in Kumbakonam, South India and confirmed with the senior Botanist. *Nigella sativa, Anethum sowa and Berberis aristata* were coarsely powdered using a mixer grinder. Equal quantities of each plant powder were mixed and subjected to aqueous and ethanol solvents for 72 hours by maceration procedure. The extract obtained was used for further studies. Two ml of the sample was subjected to phytochemical screening tests for flavonoids, phenols, carbohydrates, and proteins

The National Institute of Standards and Technology (NIST) database, which contains approximately 62,000 patterns, was used for the GC-MS study. The unknown component's spectra were compared to those of recognized components in the NIST library. The test materials' elements were identified by name, molecular weight, and structure.
Hydrogen peroxide scavenging assay, Ferric ion Reducing Antioxidant Power assay (FRAP assay) and the ABTS assay for the existence of antioxidants were done with described methods[4-5]

The herbal formulation was tested for anticancer effect by the MTT assay[8]. In a fifteen milli litre cell, trypsinized MCF 7 cells were collected and pooled. The cells were seeded then at a mass of 1105 cells per well in a medium mixture containing ten percent Fetal Bovine Serum and 1 percent antibiotic solution for 24-48 h at 37°C in a 96-well tissue culture plate. The wells in a serum-free DMEM medium were washed with Phosphate buffered saline and added with herbal formulation sample. All samples were replicated three times, and the cells were incubated in CO2 for 24 hours. Following then, MTT was applied. Cell viability was derived as follows. Cell viability % = Test OD/Control OD X 100. The herbal formulation induced apoptotic changes in MCF 7 cell line were examined by AO/PI staining. In this technique though both viable and non-viable cells absorbs AO stain which gives out green fluorescence, PI stains absorbs only by non-viable cells which gives out red fluorescence by membrane loss. IC50 concentration of herbal formulation were treated on MCF 7 cells and kept for 24h. The cells which are untreated were act as control. Then treated cells were subjected to wash by PBS and 20µl of AO/PI (100µg/ml of AO and 100µg/ml of PI) stain added and kept for 5mins. A fluorescent microscope was used to examine the stained cells

RESULTS:

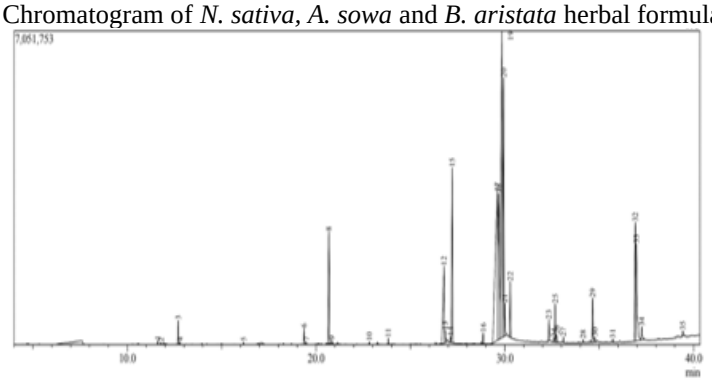
The phytochemical constituents are tabled below. (Table 1)

Table 1 showing The phytochemical constituents

Phytochemical	Aqueous	Ethanol
Carbohydrates	+	+
Protein	+	+
Alkaloids	+	+
Flavonoids	+	+
Phenol	+	+
Saponins	-	-
Terpenoids	+	+
Glycoside	+	+
Cardiac glycoside	-	+
Tannins	-	-

The final steps in the GC MS analytical process are ion detection and analysis, with compound peaks appearing as a function of their m/z ratios. The height of the peaks is proportional to the amount of each compound present. A complex sample will produce multiple peaks, with a mass spectrum as the final result. Fig 1 shows the results of GC-MS analysis of an ethanol extract of *N. sativa, A. sowa* and *B. aristata* herbal formulation which revealed a number of chemicals.

Fig 1 with GC MS Chromatogram of *N. sativa, A. sowa* and *B. aristata* herbal formulation ethanol extract



The H_2O_2 assay determines the inhibition activity of plant extracts against free radicals. The absorbance is measured at 230nm.The result depicted the increased scavenging of Hydrogen peroxide (H_2O_2) with increasing concentration of the sample. When the concentration of plant extract is increased percentage of inhibition is also increased. See Fig 2. The

maximum inhibition rate is 73.32% for aqueous and 82.56% for ethanol shown at 250 µg. The Ic50 value was found to be **173.7µg/ml** for aqueous and **115µg/ml** for ethanol**.**

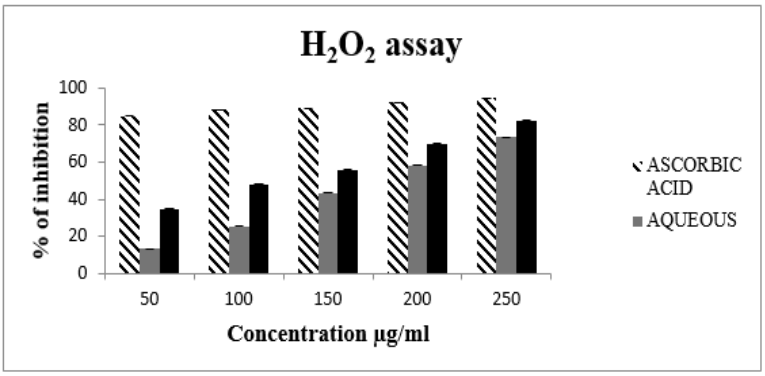
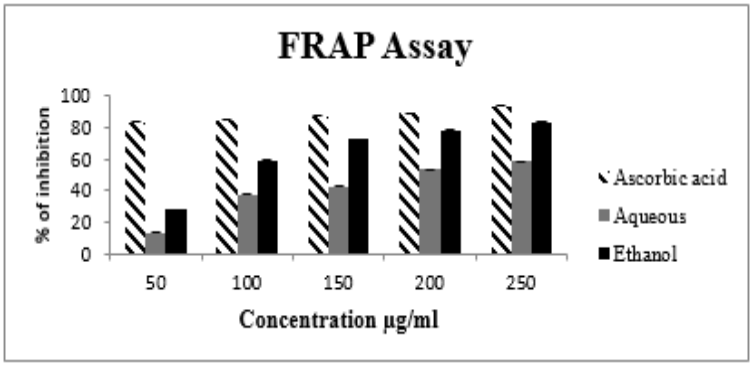


Fig 2 showing Free radical scavenging of aqueous and ethanol extract of *Nigella sativa*, *Berberis aristata* and *Anethum sowa* herbal formulation by H_2O_2 assay

In the FRAP assay, the maximum percentage of inhibition is 58.89% for aqueous and 83.77% for ethanol is seen at 250µg concentration. The IC50 value was found to be **190.6µg/ml** for aqueous and **94.7µg/ml** for ethanol. (Fig 3)
Fig 3 FRAP assay



The result depicted below the increased antioxidant effect on ABTS with increase in concentration of herbal extract. The percentage of inhibition of ABTS strongly depends on concentration of plant extract. The maximum percentage of inhibition is 60.2% for aqueous and 87.5% for ethanol is seen at 250µg concentration. See Fig 4. The decreased O.D shows the maximum of absorption. The IC_{50} concentration was 226.4µg/ml for aqueous and 133µg/ml for ethanol.

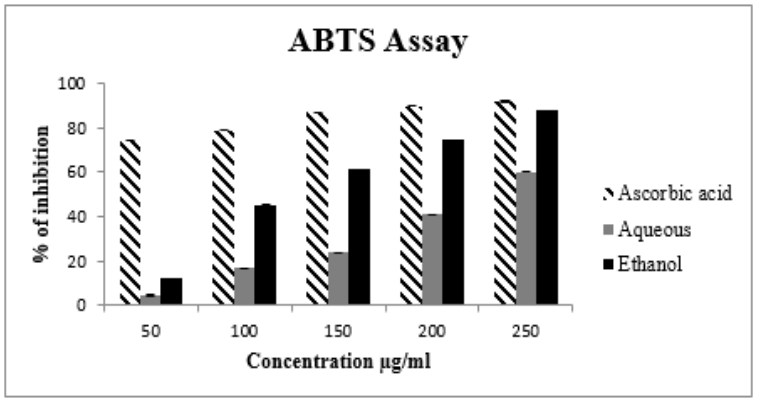


Fig 4 showing Free radical scavenging of aqueous and ethanol extract of *Nigella sativa*, *Berberis aristata* and *Anethum sowa* herbal formulation by ABTS assay.

The effects of formulation of *Nigella sativa, Berberis aristata* and *Anethum sowa* had high anticancer activity as evidenced from the MTT assay in concentration dependent manner. Formulation of *Nigella sativa, Berberis aristata* and *Anethum sowa* showed notable cell death against the MCF 7 cells. (See fig 5) Percentage of live cell was measured by MTT assay using different concentrations of the formulation of *Nigella sativa, Berberis aristata* and *Anethum sowa*. The IC_{50} value of herbal extract is found to be **181.97 µg**.

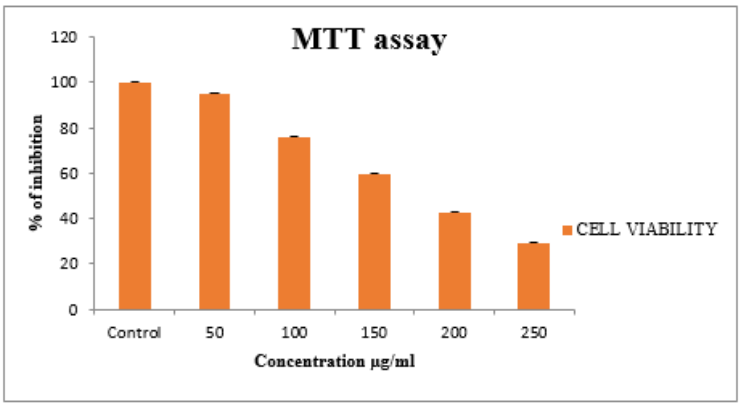
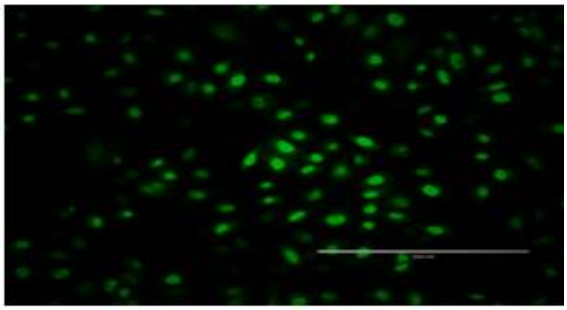


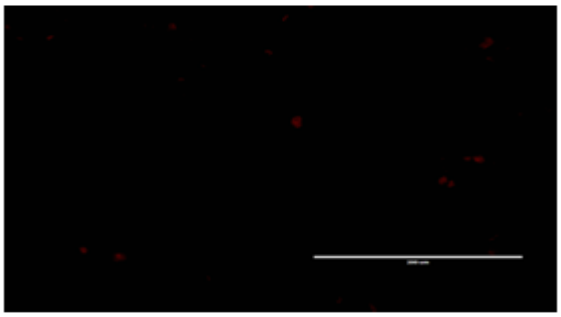
Fig 5 showing Effect of *Nigella sativa, Berberis aristata* and *Anethum sowa* herbal formulation on MCF 7 cell line by MTT assay with percentage of cell viability.

The herbal formulation extract capacity to cause apoptosis was determined in MCF-7 cell lines by AO/PI labelling method. It is to identify the morphology alterations brought on by apoptosis in breast cancer cells. Both live and dead cells can be penetrated by AO, which produces green fluorescence. PI, on the other hand, labels dead cells because of its ability to selectively enter dead cells with damaged mitochondria that produce red fluorescence. After 24 hours of treatment, signs of early apoptosis, such as membrane blebbing and nuclear condensation, were seen in the treated MCF-7 cell lines. At IC_{50} concentration, apoptotic cells increased while of viable cells decreased (see figures 6)

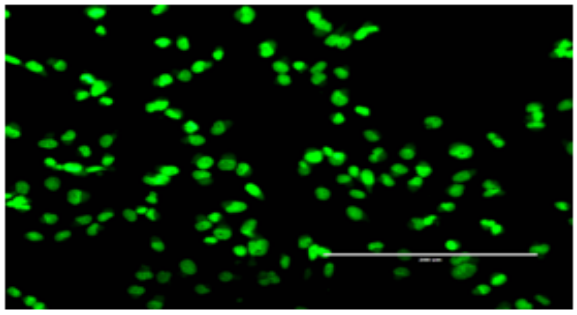
Figures 6 (a to e)



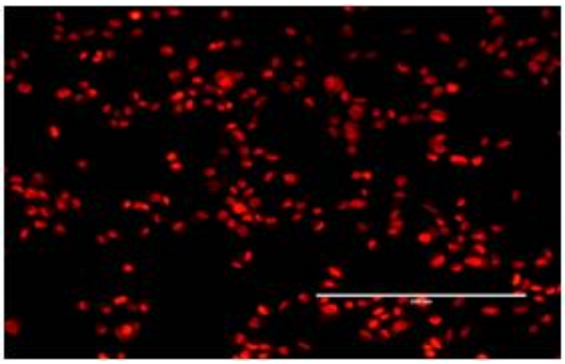
Control cells with AO stain



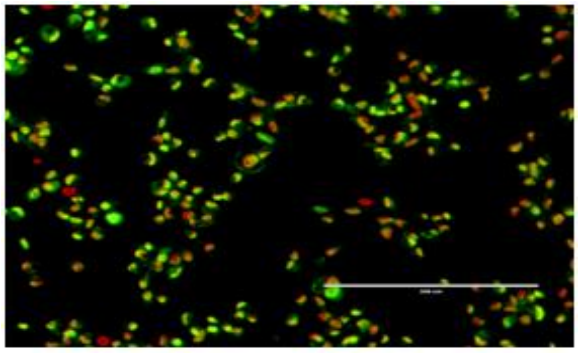
Control cells stained with PI stain



Plant extracts treated cells stained with PI stain



Plant extracts treated cells stained with AO stain



Apoptosis cell treated with AO/PI stains

DISCUSSION:

This study showed that the combined extract of the three herbs has shown antioxidant and anticancer effects. A similar result was shown in [9](Basanta Lamichhane et.al.) in *Berberis aristata* (BAE) 81.8% of inhibition is seen at 100 µg/ml and it was compared to standard L-ascorbic acid which shows 86.7% of inhibition. A similar observation was seen by another study [10] that methanolic extracts of *Anethum graveolens* seed has 78.65 % at the concentration of 500µg/ml. [10] This study shows that AG-ME has the best free radical scavenging activity.

Our FRAP assay detected antioxidant effects. A similar observation is seen by a yet another study [12] that methanolic extracts of *Anethum graveolens* [11] seed exhibit maximum absorbance of 1.387 which compared to the ascorbic value of 2.231 at 1000 µg/ml.

ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) is a water-soluble HRP substrate that yields a green end product upon reaction with peroxidase. Radical scavenging activity is explicit in the herbal extract. A similar outcome [11.12]was seen in that aqueous extract of *Anethum graveolens* seeds give out potential free radical scavenging activity at IC_{50} concentration 0f 2.68 mg/ml.

From the antioxidant results, it represents that ethanol extract has a potential antioxidant effect compared to aqueous extract. Hence ethanol extract has been taken for anticancer studies.

A similar result was shown by (Mamatha serasanambati et., al, 2015) in the *B. aristata* stem methanolic extract 50% inhibition of cell growth is seen at the 220 µg/ml concentration for 48 hours. This shows that a combined extract has more anticancer effects than individual herbs. The study has a limitation of not studying the individual effects but the effects of the mixture with individual herbs with different studies,

CONCLUSION:

Based on the results of this investigation, we conclude that the formulation of *Nigella sativa, Anethum sowa* and *Berberis aristata* herbal formulation aqueous and ethanol extracts prepared in an equal ratio demonstrated the presence of alkaloids, flavonoids, terpenoids, glycosides, and phenolic acids. H_2O_2 scavenging assay, FRAP, and ABTS assay were performed to confirm the antioxidant potential of the extracts. Combined anticancer effects are significantly more than individual extracts. Eventually, medicinal herbs can lead to therapeutic outcomes that are new. Even at high doses, the herbal extract is safe.

Conflict of interest – NIL
Financial aid - No external funding

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