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EVALUATION OF IN-VITRO ANTIOXIDANT POTENCY OF ARNEBIA NOBILIS ROOT EXTRACT

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Abstract

This study mainly deals with isolation and elucidation of structure of constituents taken from the root bark of Arnebia nobilis extract. Hydroxynaphthoquinones and isohexenylnaphthazarines mainly isolated by roots of different species of the family Boraginaceae. They were reported to have antimicrobial, anticancer and healing of wounds are main properties of these derivatives. The plant is subjected to prior screening related to phytochemical, physicochemical (IR, TLC and Mass) and pharmacological. The results showed that the plant is responsible for antioxidant properties and also the root extract have shown maximum antioxidant potency with IC50 value of 4.2 µg/ml when compared with standard ascorbic acid with IC 50 value of 4.6 µg/ml. The findings concluded that these drugs are better serving the society with reduced cost.

Keywords: *Arnebia nobilis, antioxidant, hydroxynapthoquinones, isohexenylnaphthazarines*

Introduction:

Various new products from nature and their derivatives can be used for therapeutic purpose incredibly. Every natural source act as factory of chemicals and can support many complex and unique substances synthesis having distinguished and very complex structure beyond imagination. [1]. It is well known that products from natural source has diversity in chemicals and specificity in in biochemistry and more properties related to molecular level which make them good for lead in drug discovery and can act as the basis for differentiating libraries of synthetic and combinatorial compounds [2].

The family of genus Arnebia is Boraginaceae having more amount of alkannin and shikonnin type of compounds. Ratanjot is the local name of large-scale species of Arnebia nobilis which contains rich and useful arnebin quinine [3].

It is mainly used as therapy in healing wound in impaired wounds. Naturally found hydroxynaphthoquinones and isohexenylnaphthazarines also called shikonin and alkannin are lipophilic red pigments taken from isolation of roots under species of Boraginaceae. These derivatives are mostly antimicrobial, anticancer and wound healing activity. Anthelmintic, antipyretic, antiseptic actions are associated with roots and can be used for the eye, bronchitis, abdominal pain treatment [4]. Naphthoquinones (arnebins) are the main constituents which give it efficacy and color [5].

At molecular level, metabolism of oxygen forms reactive oxygen species (ROS) including superoxide anion radical (O^{-2}), singlet oxygen ($1O_2$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (-OH). The reactive and toxic moieties reduction lead to harmful effects of oxygen [6]. Hydrogen peroxide and superoxide ion is the main reactive oxygen species source in living cells, may be formed by the mitochondrial respiration. The stress due to oxidation has major role as malignant disorders, atherosclerosis, chronic ischemia reperfusion injury, virus infection and diabetes. A pro-oxidative shift and difference in clearance of glucose occurs in diseases with oxidative stress like type-2 diabetes, neoplasm having mitochondria of muscle as site of high ROS production. Antioxidants like catalase, superoxide dismutase and vitamin like C. E and A can reverse the damage caused by oxidation [7].

Cellular oxidative stress

For the production of aerobic energy in cell, molecular oxygen is required. As a by-product of the cellular respiration, partially reduced forms of molecular oxygen, superoxide and other reactive oxygen species (ROS) are produced in cells and are referred to as ROS because of their higher reactivity than molecular oxygen. The mitochondrion is thought to be the primary intracellular source of reactive oxygen species (ROS) [8]. Furthermore, various enzymes, including peroxisomal oxidases, cytochrome P-450 enzymes, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and xanthine oxidase, are involved in the formation of cellular ROS during metabolic reactions and antibacterial defence. [9].

Nowadays, there is a growing desire to analyze the antioxidant activities of plant extracts for their safe usage in scientific research and industrial applications, thereby avoiding numerous synthetic anti-oxidants that may induce cancer. Phenolic compounds have been discovered to be powerful antioxidants that can scavenge or suppress ROS and RNS creation by blocking or chelating metals implicated in free radical production, as well as upregulate or protect anti-oxidant defence, avoiding carcinogenesis [10]. The goal of this study is to determine the antioxidant capacity of Arnebia nobilis root extracts in vitro.

Materials and methods

Plant material

Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR, verified the root bark sample collected located in Chandni Chowk Khari Baoli Street, in New Delhi. Following that, a voucher specimen number NISCAIR/RHMD/Consult/2016/2969/162 was placed in the department RHMD of NISCAIR.

Preliminary screening of phytochemicals

Components of plants were kept for drying and ground with grinders, then sieved to achieve the fine powder. In separate test tubes, one gram leaf which is in powdered form and includes stems also for maceration in 10 ml solvents, including DMSO, Water, Chloroform, Ethanol, Ethyl acetate, Dichloromethane,

Petroleum Ether, n-Hexane, Acetone, Methanol, Methanol-Water (70:30), BAW (n-Butanol-Acetic Acid-Water; 4:1:5), and Ethyl Me With a cotton plug, the powdered medication mixture and solvents were sealed and were kept aside almost 2days. Cotton was taken out and the mixture was filtered. Qualitative tests were performed on the filtered filtrate to identify different phytoconsitituents like flavonoids, alkaloids, etc.

Mass Extraction

250g of Amebia nobilis root powder was cold macerated for 7 days at room temperature with 400ml ethyl methyl ketone. It was filtered after 7 days, and the filtrate was concentrated over a water bath to produce a reddish brown residue. The filtered residue was then subjected to a qualitative test to determine the presence of flavonoids, alkaloids, glycosides, terpenoids, carbohydrates, proteins, and tannins, among other phytoconstituents.

Phytochemical Screening of Extracts

Fractionation

The concentrated root extract (40mg) separately kept in china dish in which silica gel was then added with mesh 200very slowly with mixing continuously with help of spatula made of steel in quantity of 800mg and a desired consistency is obtained. This was then dried and big size lumps were broken for smooth flow of mixture.

The column with length of 5 ft and dimension of diameter internal as 16mm was kept for process and dried which was plugged with absorbent cotton at lower part. This was then filled with n-hexane almost half quantity. The prepared silica gel in very less quantity were taken and poured and made to gently settle down up till required length is obtained. The root extract containing slurry of silica gel was poured separately in column and eluted with various types of solvents, in the order of n-hexane (10), n-hexane : chloroform (75:25), n-hexane : chloroform (50:50), n-hexane : chloroform (25:75), chloroform (100), chloroform : toluene (75:25), chloroform : toluene (50:50), chloroform : toluene (25:75), toluene (100), toluene : ethyl acetate (75:25), toluene : ethyl acetate(50:50), toluene : ethyl acetate (25: 75), ethyl acetate (100), ethyl acetate : methanol (75:25), ethyl acetate : methanol (50:50), ethyl acetate : methanol (25:75), methanol (100), methanol: formic acid (50:50), methanol : formic acid : water (4:1:5). Nineteen fractions were collected in a conical flask and marked. The marked fractions were subjected to TLC to check homogeneity of various fractions [5]. Chromatographically identical various fractions (having same Rf values) were combined together and concentrated.

Physiochemical characterization

Following TLC, four bioactive components were extracted from column chromatography and identified by qualitative chemical analysis. TLC, IR, and mass spectroscopy were used to characterise the samples.

TLC Evaluation

Individual phytoconstituents were identified by performing special TLC analysis using toluene: formic acid (8.5:1.5) as mobile phase for each category of phytoconstituents that had already been identified with qualitative chemical analysis.

Mass Spectral Analysis

At Jubiliant Chemsys in Greater Noida, electrospray mass spectra for the isolated chemicals were recorded on a Thermo Finnegan LCQ Advantage max ion trap mass spectrometer. Through a Finnegan Surveyor auto sampler, the 10l samples (dissolved in a solvent such as methanol/DMSO) were delivered into the ESI source. Ms pump pumped the mobile phase (90:10 MeOH/ACN: H2O) at a rate of 250 l/min. The ion spray voltage was set to 5.3 kV, while the capillary voltage was set to 34 volts. The MS scan takes up to 2.5 minutes, and the spectra printouts in TIC are an average of more than 10 scans at peak top.

Evaluation/pharmacological screening

Acute toxicity studies

Requirements

Females should be nulliparous and non-pregnant when choosing an animal species. Each animal should be between the ages of 8 and 12 weeks when dosing begins.

Housing and dietary requirements: 22°C + 3°C is a temperature of 22°C plus 3°C.
Volume should not generally exceed 1mL/100g of body weight for preparing dosages. CPCSEA guidelines (NO. IAEC/KSOP/E/15/001) were used to approve all of the protocols.

1. A single dosage of the test material is given through gavage using a stomach tube or an appropriate intubation cannula. In the unlikely event that a single dose is not achievable, the dose may be given in smaller fractions throughout a 24-hour period.
2. Prior to dosage, animals should be fasted (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water should be withheld for 3-4 hours). The animals should be weighed and the test material administered after the fasting interval. Food can be withheld for another 3-4 hours in rats or 1-2 hours in mice after the chemical has been provided. Depending on the length of the period, it may be essential to provide food and water to the animals when a dose is given in fractions over time.

Procedure
For each phase, three animals are employed. The beginning dose is chosen from one of four predetermined levels: 5, 50, 300, or 2000 mg/kg body weight. The beginning dose level should be the one that is most likely to result in death in at least some of the dosed animals. The flow charts in Annex 2 of the OECD standards outline the steps to take for each of the starting doses. A limit test should be performed when available data suggests that fatality at the maximum beginning dose level (2000 mg/kg body weight) is unlikely. When there is no information on a chemical to be tested, the beginning dose of 300 mg/kg body weight is advised for animal welfare considerations. The development, duration, and severity of toxic symptoms dictate the time gap between treatment groups. Treatment of animals with the following dose should be postponed until the prior dosed animals have been confirmed to have survived.

Individual animals are observed after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, unless they are removed from the study and humanely killed for animal welfare reasons or are found dead. The appearance and disappearance of toxicity signals must be timed carefully, especially if toxic indicators have a tendency to be delayed.

***In-vitro* antioxidant assay**
Free radicals of various types are constantly created in biological systems to meet specific metabolic needs. When the production of these species surpasses the antioxidant mechanism's capacity, they cause significant damage to cells, resulting in oxidative damage to tissues and macromolecules, which finally leads to disease, particularly degenerative disorders and widespread lysis [11]. Enzymes like superoxide dismutase, glutathione peroxidase, and catalase, as well as endogenous antioxidants like -tocopherol, ascorbic acid, -carotene, and uric acid, protect the living system from this. Because endogenous antioxidants that protect cells from free radical damage and extensive lysis are not 100 percent effective at scavenging and reducing the formation of oxygen-derived species, micronutrients or antioxidants taken as supplements are especially important in reducing cumulative oxidative damages.

DPPH radical Scavenging activity [12]
Materials
All the chemicals like DMSO, ascorbic acid etc. are procured from CDH Fine Chemicals. The chemicals are in good packing condition to avoid the surrounding interruption because it may manipulate our readings. The DPPH is a light sensitive drug, so handling it is very important otherwise the drug may damage itself by absorbing light.

Method
The bleaching of the purple-colored ethanol-solution of DPPH was used to determine the hydrogen atom of the resulting compounds' electron donating capacities. A 0.1mM DPPH solution in ethanol was produced, and 0.1ml of this solution was added to 3ml of the test drug at various concentrations (1, 2, 4, 8, 16, 32g/ml). The absorbance was measured against a blank at 517nm using a UV-visible double beam spectrophotometer after 30 minutes of incubation at room temperature (Shimadzu-1800). The percent scavenging activity of test medicines was evaluated at various doses, and their IC50 values were compared to those of ascorbic acid, which was employed as the

standard. The reaction mixture's lower absorbance implies greater free radical scavenging activity. The extract's antioxidant activity was measured using the IC50 method. The IC50 value is the concentration of a test medication (in g/ml) that suppresses the production of DPPH radicals by 50%.

Hydrogen peroxide scavenging (H_2O_2) assay [13]

Material

All of the chemicals were analytical quality, and all of them were obtained from CDH Lab. in New Delhi, including hydrogen peroxide and ascorbic acid. The chemicals are in good packing condition to avoid interference from the environment, which could skew our results. Because hydrogen peroxide is a light-sensitive medicine, special care must be taken when handling it; otherwise, the drug may cause damage to itself by absorbing light.

Method

In phosphate buffer, make a 40 mM hydrogen peroxide solution (50 mM pH 7.4). A spectrophotometer was used to determine various concentrations of hydrogen peroxide using absorbance at 230 nm. The test drug was mixed with hydrogen peroxide at various concentrations (1, 2, 4, 8, 16, and 32g/ml) in distilled water, and absorbance at 230 nm was measured after 10 minutes using a UV-visible double beam spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide (Shimadzu-1800). The percent scavenging activity of test medicines was evaluated at various doses, and their IC50 values were compared to those of ascorbic acid, which was employed as the standard. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\%\ \text{scavenged}\ (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

Where A_i is the absorbance of control and A_t is the absorbance of test.

Phosphomolybdenum method [14]

Material

All the chemicals were analytical grade and all chemicals are procured from CDH Lab., New Delhi. The chemicals are in good packing condition to avoid the surrounding interruption because it may manipulate our readings.

Method

By combining sample of 0.1 mL (100 g) diluted to get solution with 1 mL of reagent, total antioxidant capacity can be measured (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube was sealed and incubated for 90 minutes in a boiling water bath at 95°C. The absorbance of the aqueous solution was measured at 695 nm using a UV-visible double beam spectrophotometer after the sample was cooled to room temperature (Shimadzu-1800). The percent scavenging activity of test medicines was evaluated at various doses, and their IC50 values were compared to those of the standard which is ascorbic acid..

Fenton's reagent method [15]

Material

All chemicals were analytical grade and all chemicals are procured from CDH Lab, New Delhi. The chemicals are in good packing condition to avoid the surrounding interruption because it may manipulate our readings.

Method

According to the Fenton's reagent method, a combination of Fe2+ and H202 indicates oxidation of organic molecules in diluted aqueous solution. 0.5M H2SO4 was used to modify the pH of a 50ml sample to 3-4. The UV spectrophotometer was used to get absorbance which is being measured at 532 nm after 50 L of saturated aq. ferrous ammonium sulphate [Fe (NH4)2(SO4)2] was introduced, followed by 200 L of 30% H202 (Shimadzu-1800). The percent scavenging activity of test medicines was evaluated at various doses, and their IC50 values were compared to those of the standard which is taken as ascorbic acid.

Statistical data analysis

Graph pad prism 3.0 which is software by san Diego, CA, is used for getting results of statistical analysis All results were expressed as mean ± standard error of mean (S.E.M). Groups of data were compared with the analysis of variance (ANOVA) followed by Dunnett's test. Values were considered statistically significant when $p<0.05$ and $p<0.01$.

Results and discussion

Acute toxicity studies

The animals showed no mortality in acute toxicity study and the LD_{50} cut off was identified as 2000mg/kg body weight.

Preliminary Phytochemical Screening

Table 1 shows the preliminary phytochemical screening results.

The preliminary phytochemical screening of powdered root of arnebia nobilis showed the presence of alkaloids, glycosides, and flavonoids enriched with the solvent ethyl methyl ketone.

Mass Extraction

Cold maceration carried out to obtain the yield in total as 31.51gm and 40 % as the percentage yield.

Isolation and preliminary identification of biologically active new leads from Arnebia nobilis

Elution carried out in column and 4 different types of fractions were obtained with root extract of *Arnebia nobilis*, fraction4(n-hexane-chloroform) (25:75)-*[F4]*, fraction 11(Toluene-Ethyl acetate) (50:50)-*[F11]*, fraction 3(Toluene-Ethyl acetate) (25:75)–*[F3]*and fraction 4(Ethyl Acetate-Methanol) (75:25)-*[F4]*. The phytochemical screening of this fraction was done and results are shown in table 2.

Physico-chemical characterization of bioactive lead from Arnebia nobilis

The best derivatives act as lead molecules, F1, F2, F3 and F4 were processed for characterization by Mass spectroscopy. The results show that the prepared compounds are pure.

Invitro antioxidant activity from *Arnebia nobilis*

Hydrogen peroxide Scavenging Assay

The IC_{50} value of the test compound Arnebia nobilis root extract giving result as 2-8 µg/ml comparing it with standard taken as ascorbic acid having IC_{50} as 2.0 µg/ml. The results are expressed in table 7 and figure 6.

DPPH Assay

The IC_{50} value of the test compound Arnebia nobilis root extract shows value of 4.8µg/ml as comparing with standard taken as ascorbic acid showing IC_{50} = 4.2 µg/ml.

The results are expressed in table 8 and figure 7.

Total antioxidant capacity by Phosphomolybdenum Method

The IC_{50} value of test compound Arnebia nobilis root extract was found to be 2.7µg/ml as compared with standard ascorbic acid (IC_{50} = 2.2µg/ml). The results are expressed in table 9 and figure 8.

Fenton's Method

The IC_{50} value of test compound Arnebia nobilis root extract showing the result as 4.2µg/ml as comparing it to the standard taken as ascorbic acid with IC_{50} = 4.6µg/ml. The results are expressed in table 10 and figure 9.

The existence of Napthoquinone, glycoside, flavonoid, and isoquinoline alkaloid in the bioactive ingredients extracted from the roots of Arnebia nobilis was clearly indicated by their characterization of structure prediction by Mass spectral analysis.

Through Mas spectral analysis and interpretation of spectra, the presence of four bioactive constituents in root extract, namely Arnebin-1, Isorhamnetin, Diosmetin, and Lindechunine A, is revealed in the table of Mass spectra, with the corresponding (M+1)+ peak (m/z 371), M+ peak (m/z 316), M+ peak (m/z 300), and (M-1)- peak (m/z 350). Arnebia-1 was already found in large amounts in the same plant, as indicated by previous literatures, whereas the other bioactive ingredients were extracted and published for the first time from Arnebia nobilis root extract.

With all methodologies such as hydrogen peroxide scavenging test, DPPH assay, phosphomolybdenum assay, and Fenton's assay, the above findings of the research investigation clearly demonstrated that the root extract of Arnebia nobilis contain significant antioxidant activity in-vitro. Furthermore, when compared to conventional ascorbic acid, the test compound root extract has showed the highest antioxidant potency in Fenton's experiment. Arnebia nobilis root extract's bio fractionated isolate, Diosmetin (a flavonoid), may be a likely active constituent responsible for antioxidant activity, and it, along with other active constituents like Arnebin-1,

Isorhametin, and Lendichunin A, may be synergistic in response for potent antioxidant activity. In the near future, this has to be investigated further with specific mechanism-based studies of particular bioactive elements.

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References

[1] Arora A., Identification and characterization of Ratanjot (arnebia nobilis Reichb.f). Natural Product Radiance 2009;8(2):142-145.

[2] Coker PS, Radecke J, Potato disc tumor induction assay: a multiple mode of drug action assay. Phytomedicine. 2003;10(2-3p):33-8.

[3] Fotakis G, Timbrell JA, In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicology letters, 2006;5;160(2):171-7.

[4] Ferreira GM and Laddha KS, A new method for the isolation shikalkin from the roots of *Arnebia nobilis* Reich.f. Indian Journal of Natural Product and Resources, 2013;4(3):270-272.

[5]. Arora A & Rastogi D, Dyeing parameters of hydroxynaphthoquinones extracted from Arnebia nobilis Rech.f. Indian Journal of Fibre & Textile research. 2012;37;91-97.

[6] Kaushik D, Roychoudhury A, Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science. 2014; 2;1-53. doi:10.3389/fenvs.2014.00053.

[7] Kurutas EB, The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal. 2016;15(1):71. https://doi.org/10.1186/s12937-016-0186-5.

[8] Singh S, Srivastava RK, Srivastava M, Puri SK and Srivastava K. *In-vitro* culture of *Plasmodium falciparum*: Utility of Modified (RPNI) Medium for drug sensitivity studies using SYBR Green I assay. Exptl. Parasitol. 2011; 127: 318-321.

[9] Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS Sources in Physiological and Pathological Conditions. Oxidative medicine and cellular longevity, 2016, 1245049. https://doi.org/10.1155/2016/1245049.

[10] Tan, B. L., Norhaizan, M. E., Liew, W. P., & Sulaiman Rahman, H. (2018). Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Frontiers in pharmacology, 9, 1162. https://doi.org/10.3389/fphar.2018.01162.

[11] Nagarajan K, Mazumder A and Ghosh LK, Search towards an insight for comparative anti-tumour effects of Wrightia tomentosa leaf & bark in ehrlich ascites carcinoma bearing mice. 2008 31;8(4):408–15. http://dx.doi.org/10.3742/OPEM.2008.8.4.408.

[12] Ichikawa K, Sasada R, Chiba K and Gotoh H, Effect of Side Chain Functional Groups on the DPPH Radical Scavenging Activity of Bisabolane-Type Phenols. Antioxidants. 2019;8;65. https://doi.org/10.3390/antiox8030065.

[13] Fernando CD, Soysa P, Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H2O2) scavenging activity of plant extracts. MethodsX. 2015;2:283–291. doi:10.1016/j.mex.2015.05.001.

[14] Abdille MH, Singh RP, Jayaprakasha GK and Jena BS, Antioxidant activity of the extracts from Dillenia indica fruits. 2005; 90(4):891–896. doi:10.1016/j.foodchem.2004.09.002.

[15] Prakash, Dhan P, Upadhyay G, Pushpangadan P and Gupta C, Antioxidant and Free Radical Scavenging Activities of Some Fruits. Journal of Complementary and Integrative Medicine. 2011;8(1);1-16. https://doi.org/10.2202/1553-3840.1513.

Table 1: Prileminary phytochemical screening of arnebia nobilis root powder

	ALKALOIDS		GLYCOSIDES				FLAVONOIDS		VITAMINS		
	Dragendroff test	Hagers test	Borntragers test	Mod. borntragers test	Legal test	Baljet test	Ammonia test	Sulphuric acid test	Vitamin A test	Vitamin D test	Vitamin C test
DMSO	-	++	++	++	-	-	-	+	-	-	-
n-hexane	++	+	-	+	-	+	+	++	-	-	+
Pet.ether	+	+	-	+	-	+	++	++	-	-	+
Chloroform	+	++	++	-	-	+	+	-	-	-	+
Dichloromethane	++	-	-	++	-	++	+	-	-	-	-
Acetone	-	-	++	-	-	++	-	-	-	-	-
Ethyl acetate	++	+	++	+	-	+	+	+	-	-	-
Methanol	+	++	++	-	+	+	++	++	-	-	-
Ethanol	++	-	++	+	-	+	-	-	-	-	-
Water	+	++	-	-	-	+	+	+	-	-	+
Methanol+water	-	+	+	-	-	-	-	+	-	-	+
BAW	++	-	+	+	-	+	-	-	-	-	-
Ethyl methyl ketone	-	++	++	+	+	++	++	++	-	-	-

(++) - Presence of huge active constituents
(+) - Presence of moderate active constituents
(-) - Absence of active constituent

Table 2: phytochemical screening of isolated fractions of arnebia nobilis root extract

SCREENING RESULT FOR ISOLATED FRACTIONS

	ALKALOIDS		GLYCOSIDES				FLAVONOIDS		VITAMINS		
	Dragendroff test	Hagers test	Borntragers test	Mod. borntragers test	Legal test	Baljet test	Ammonia test	Sulphuric acid test	Vitamin A test	Vitamin D test	Vitamin C test
FRACTION 1	++	+	+	++	-	+	-	-	-	-	-
FRACTION 2	++	++	+	+	-	+	-	+	-	-	-
FRACTION 3	++	+	-	++	-	++	-	+	-	-	-
FRACTION 4	++	++	-	++	-	+	+	++	-	-	-

Table 3: fraction1 (f4) of arnebia nobilis

Mass	Ion	Product ion and composition of the neutral particle lost	Corresponding peak with m/z value	Substructure or compound type
1	-	$[M+1]^+$	371	Molecular ion with protonated ion peak
17	OH^+	$[M-17]^+$ (OH)	353	N - oxides
19	H_3O^+	$[M-19]^+$	351	Abundant ion in M-19 (base peak)
67	$C_5H_7^+$,$C_4H_3O^+$	$[M-67]^+$	303	Furyl Ketones
68	$C_5H_8^+$, $C_4H_4O^+$	$[M-68]^+$ (C_5H_8) (C_4H_4O)	302	Cyclohexenes Cyclohexenones
87	$C_5H_{11}O^+$	$[M-87]^+$	283	Alcohols,ethers,esters 'O' indicators
88	$C_4H_8O_2^+$	$[M-88]^+$	282	α – methyl- methyl esters
131	$C_{10}H_{11}^+$	$[M-131]^+$	239	Tetralins

Table 4: fraction 2 (f11) of arnebia nobilis

Mass	Ion	Product ion and composition of the neutral particle lost	Corresponding peak with m/z value	Substructure or compound type
-	M^+ molecular ion	-	316	Molecular ion of glycoside type
34	$(OH)_2^+$	$[M-34]^+$ (OH+OH)	282	-
35	-	$[M-35]^+$ ($OH+H_2O$)	281	2× 'O' Indicator as base peak
36	C_3^+	$[M-36]^+$ (H_2O+H_2O)	280	2× 'O' Indicator
83	$C_6H_{11}^+$ $C_5H_7O^+$	$[M-83]^+$ $[M-83]^+$	233	Alkenes, Alicyclic, Cycloalkanones.

Table 5: fraction 3 (f12 &f13) of arnebia nobilis

Mass	Ion	Product ion and composition of the neutral particle lost	Corresponding peak with m/z value	Substructure or compound type
-	M^+	-	300	Molecular ion of flavonoid type
21	$C_2H_2O^{++}$	$[M-21]^+$	279	-
43	$C_3H_7^+$ $C_2H_3O^+$ CH_3CO^+	$[M-43]^+$ (C_3H_7) $[M-43]^+$ (CH_3CO)	257	Alicyclics, cycloalkanones,cycloalkanols, Aromatic methyl ethers.
44	$C_2H_4O^+$ CO_2^+	$[M-44]^+$(C_2H_4O) $[M-44]^+$(CO_2)	256	Cycloalkanols, cyclic ethers, Lactones.
88	$C_4H_8O_2^+$	$[M-88]^+$	212	α-methyl-methyl esters
105	$C_8H_9^+$ $C_7H_5O^+$	$[M-105]^+$	195	Alkyl aromatics Benzoyl derivative.

Table 6: fraction 4 (f14) of arnebia nobilis

Mass	Ion	Product ion and composition of the neutral particle lost	Corresponding peak with m/z value	Substructure or compound type
1	-	$[M-1]^-$	350	Occurs for moderately basic & acidic compound
31	CH_3O^+ 'O' Indicator	$[M-31]^+$ (CH_3O)	320	Methyl ethers and primary alcohols.
50	$C_4H_2^+$	$[M-50]^+$	301	Aromatics
72	$C_4H_8O^+$ $C_4H_{19}N^+$	$[M-72]^+$	279	Alkanones- 'O' Indicator Alkenes- 'N' Indicator

Table 7: Hydrogen peroxide Scavenging Assay Results

S. No.	Compound name	% inhibition (µg/ml)						IC50 (µg/ml)
		1	2	4	8	16	32	
1.	Test compound (Arnebia nobilis)	85.84	87.51	88.15	88.80	94.98	100	**2.8**
2.	Standard compound (Ascorbic Acid)	85	85.78	86.84	90	91.84	100	**2.0**

Table 8: DPPH Assay Results

S. No.	Compound name	% inhibition (µg/ml)						IC50 (µg/ml)
		1	2	4	8	16	32	
1.	Test compound (Arnebia nobilis)	41.17	47.05	76.47	79.41	88.23	100	**4.8**
2.	Standard compound (Ascorbic Acid)	47.54	47.54	50.81	75.40	93.44	100	**4.2**

Table 9: Antioxidant capacity by Phosphomolybdenum Method Results

S. No.	Sample (compound name)	Inhibition in percent (µg/ml)						IC50 (µg/ml)
		1	2	4	8	16	32	
1.	Test compound (Arnebia nobilis)	106.96	105.47	104.47	102.98	101.99	100	**2.7**
2.	Standard compound (Ascorbic Acid)	109.47	108.53	107.58	106.63	104.26	100	**2.2**

Table 10: Fenton's Method Results

S.no.	Compound name	% inhibition (µg/ml)						IC50 (µg/ml)
		1	2	4	8	16	32	
1.	Test compound (Arnebia nobilis)	103.60	103.27	102.29	101.63	100.98	100	4.2
2.	Standard compound (Ascorbic Acid)	94.57	96.00	96.57	97.14	99.92	100	4.6

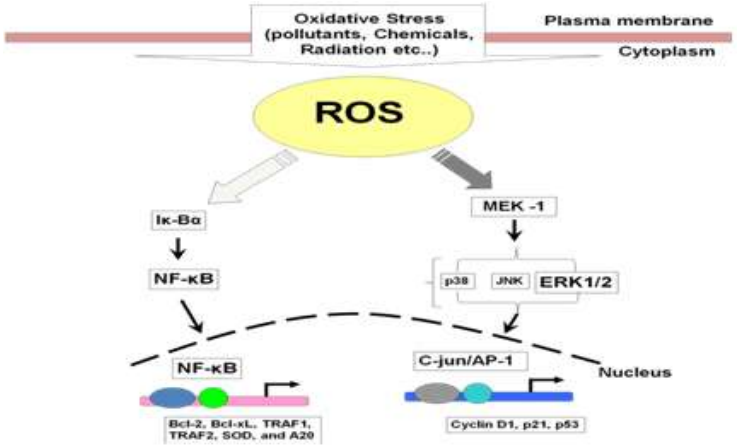

Figure 1: Oxidative stress and cell signaling.

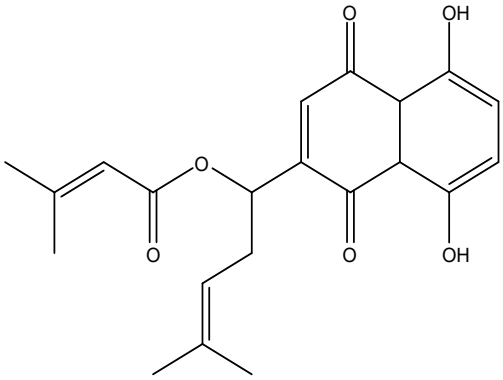

Figure 2: Arnebin-1
Final structure of the compound F1
Arnebin-1
Mol. Weight: 370
Molecular formula: $C_{21}H_{22}O_6$
Naphthoquinone derivative

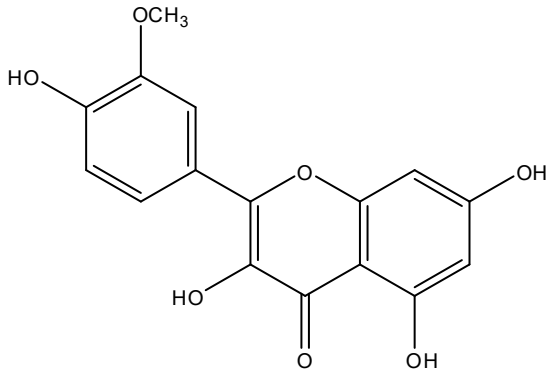

Figure 3: Isorhamnetin

Final structure of compound F2.

Isorhamnetin

Mol. Weight: 316

Mol. Formula: $C_{16}H_{12}O_7$ (Glycoside)

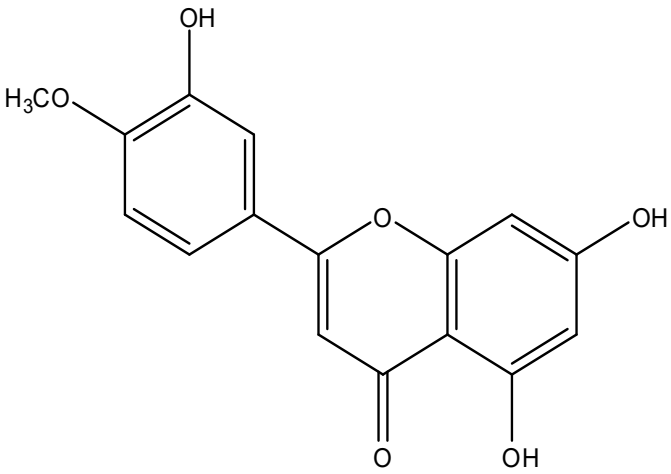

Figure 4: Diosmetin

Final structure of the compound F3

Diosmetin (Flavonoid)

Mol. Weight: $C_{16}H_{12}O_6$

Mol. Weight: 300

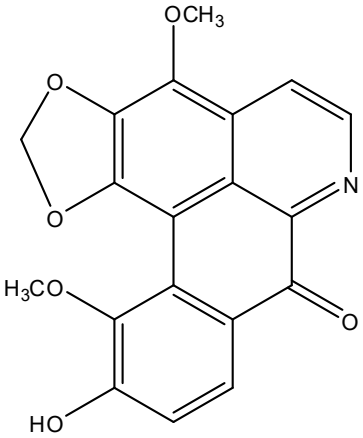

Figure 5: Lindechunine A

Final Structures of compound F4:

Lindechunin A (Isoquinoline alkaloid)

Mol.weight: 351

Mol. Structure: $C_{19}H_{13}NO_6$

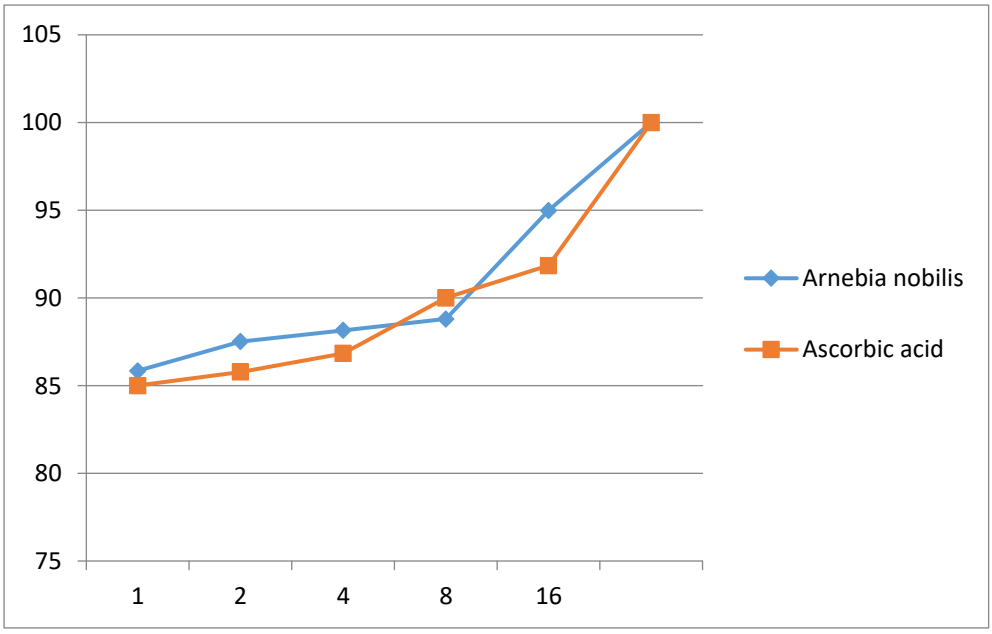

Figure 6: Hydrogen peroxide Scavenging Assay

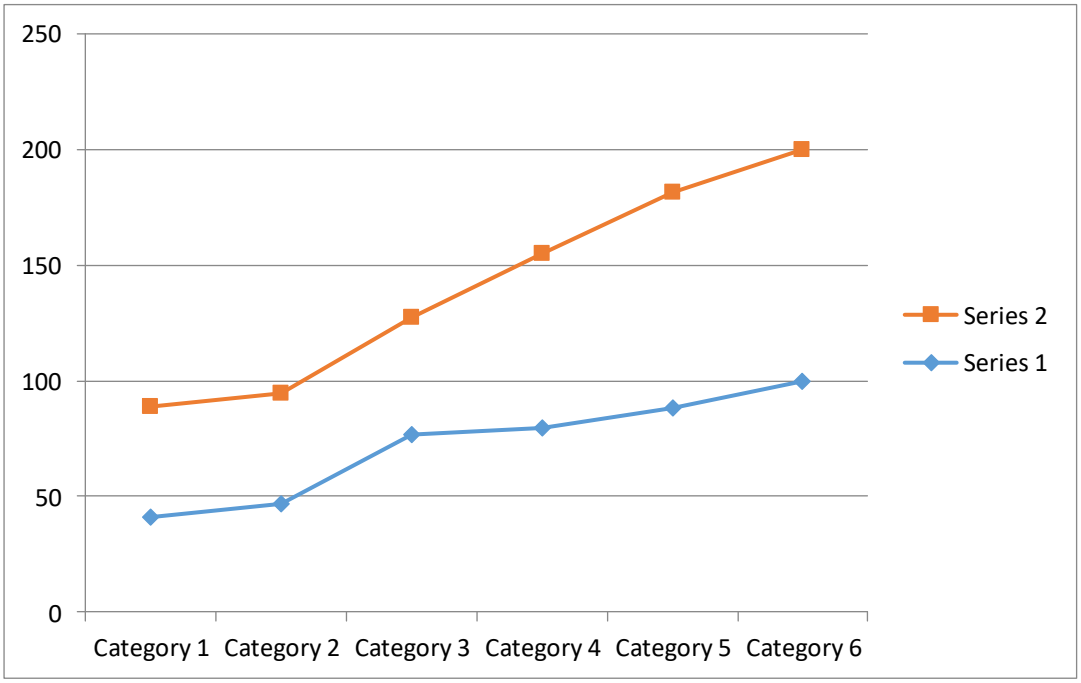

Figure 7: DPPH Assay

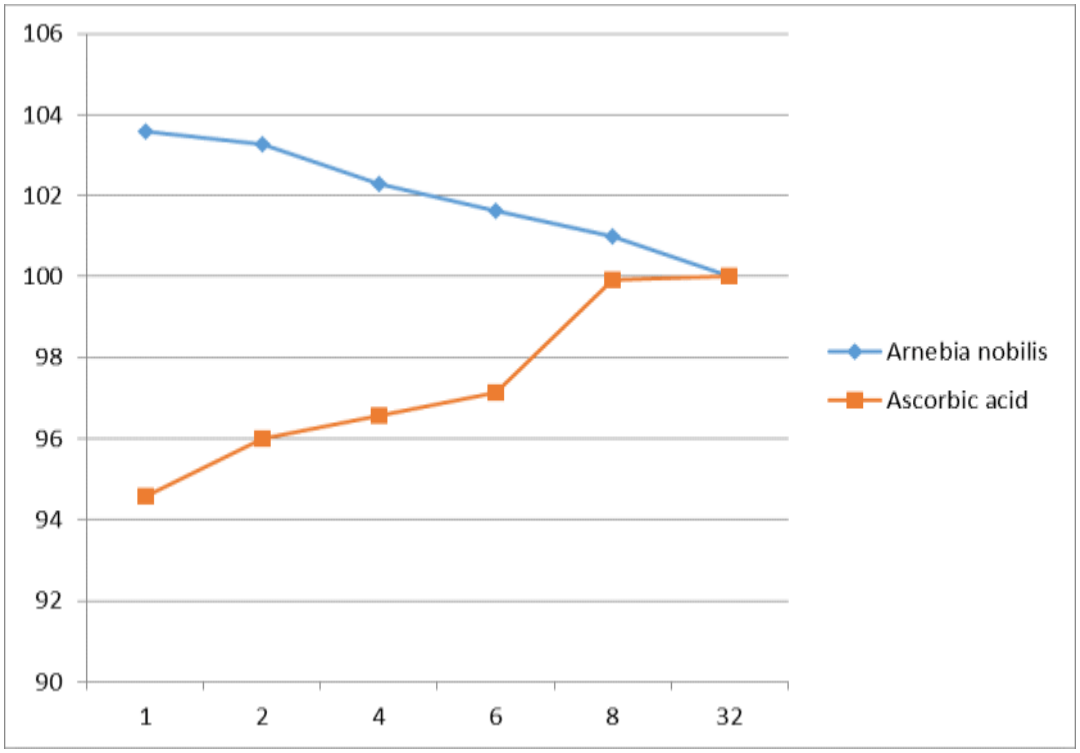

Figure 8: Antioxidant capacity by Phosphomolybdenum Method Results

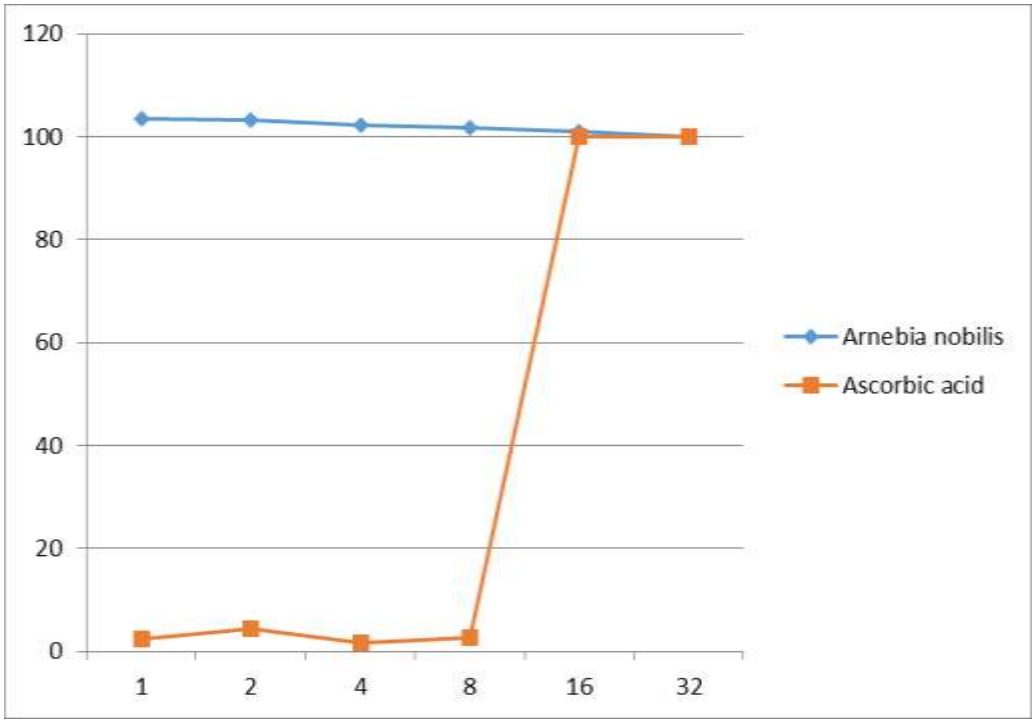

Figure 9: Fenton's Method Results