PREPARATION AND EVALUATION OF *VINCA ROSEA* ENRICHED FRUIT BASED BEVERAGES

Dissertation I Report

Submitted by

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CERTIFICATE

This is to certify that Ankit Choudhary has personally completed M.Sc. Pre-dissertation entitled, "***Preparation and evaluation of Vinca rosea enriched fruit based beverages***" under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of pre-dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfilment of the conditions for the evaluation leading to the award of Master of Food Technology.

Signature of Supervisor

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DECLARATION

I hereby declare that the work presented in the pre- dissertation report entitled "Preparation and evaluation of Vinca rosea enriched fruit based beverages" is my own and original. The work has been carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India under the guidance of Dr.Vikas Kumar**,** Assistant Professor (Food Technology) of School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Food Technology.

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I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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Chapter 1: INTRODUCTION

From ancient times, people are using creepers, shrub, herbs and trees for medicinal and ornamental purposes and *Catharanthus roseus* is one of them, which is also known as Vinca Rosea, wild and endangered species. It is also known by different names like periwinkle, Ammocallis rosea and Lochnera rosea depending on the location and language (Barik et al, 2016). Table 1 lists down the different names of C. roseus according to varying languages.

Catharanthus is upto 1 m tall herb, whose flowers vary from pink to purple in color (Mishra and Verma 2017). *Catharanthus roseus* belongs to Apocynaceae family and the word 'Catharanthus' is a greek word means "pure flower" in Greek and "roseus" means red or rosy. *Catharanthus roseus* belongs to kingdom Plantae, Division- Magnoliophyta, Class- Magnoliopsida, Order- Gentianales, Genus- catharanthus, Species- C. roseus. People use this crop because of its pharmaceuticals property such as Antidiabetic property, anticancer property (Balaji et al, 2014), antimicrobial property, antioxidant property, antihelmintic property, antidiarrheal, antiulcer property, hypertensive property, phytoremediation, antimutagenic, antimitotic property,memory enhancement activity, hypolimidic effect,wound healing property,blood cleanser, antihypercholesterolemic activity, disinfectants, Transquilizing and sedative action. There is a wide use of this plant in different parts of the world. In India the extract of plant leaves is used as application of wasp sting. In Africa plant leaves are used for rheumatism and menorrhagia (Sain et al, 2013). In Hawai people use boiled plant extract to stop bleeding (Sain et al, 2013). In Mauritius juice extract of plant leaves is used to cure indigestion and dyspepsia problem (Sain et al, 2013). In West Indies and Nigeria Vinca rosea plant is used to cure diabetes (Sain et al, 2013). In Cuba and Jamaica flower extract is used for eye washing of infants (Sain et al, 2013). In Brahamas concentrated extract of vinca rosea is used to cure asthma, flatulence and tuberculosis (Sain et al, 2013). In Malaysia it is used to treat diabetes, cancer, insomnia and hypertension (Sain et al, 2013). In America extract of plant is used to cure sore throats, chest ailments, and laryngitis by gargling (Sain et al, 2013). In Madagascar plant leaves and roots are used for laxative effect, curing toothache, detoxification and anthelmintic (Sain et al, 2013). In Philippines concentrated plant juice used to cure diabetes, concentrated juice of young plant leaves to

cure stomach cramps, concentrated extract of root is used for dysentery and crude extract of leaves and roots is having anticancer property (Sain et al, 2013).

Chapter 2: PROBLEM BACKGROUND

Diabetes is a life threatening metabolic disorder which is caused due to disturbance in the metabolism of protein, carbohydrates, fat. It occurs when there is a deficiency in the production of insulin by the pancreas in the beta cells of the pancreas (Yadao et al, 2015). There is no effective medicine available in the market till date to cure diabetes mellitus and due to this people are shifting towards the traditional natural ayurvedic products to minimize the side effects associated with the use of insulin and oral hypoglycemic agents (Balaji et al, 2014). Past researches have proved that *Catharanthus roseus* has antidiabetic property owing to the presence of vincristine and vinblastine (Modak et al, 2006; Gupta et al, 1998). It has also been proved that ethanolic extracts of flowers and leaves of this plant lower the blood glucose level to a greater extent as compared to standard drug gliben clamide (Sharma et al, 2013).

Chapter 3: REVIEW OF LITERATURE

Origin and Distribution

The origin of *Catharanthus roseus* is from islands of Madagascar (Mishra et al, 2017; Aruna et al, 2015).It is widely distributed in different subtropical and tropical area of the world along United States (Aruna et al, 2015). It is present in every continent except Antartica or some islands. Therefore it is available throughout the year except winters and hence in temperate regions because their growth is inhibited in low temperature. It is also cultivated in gardens and widely distributed in the wasteland (Qureshi et al, 2008).

Nutritional Composition

From past few decades, *Catharanthus roseus* has been reported to be a perfect blend of ornamental and medicinal plants which have witnessed its utilization in the traditional medicinal systems. Currently, *Catharanthus roseus* has been found to have immense potential in food industry also. To know the nutritional significance of any edible plant, the proximate and nutritional composition analysis is important. The plant and its different parts i.e. root, shoot, leaves and flower comprises of wide range of physicochemical and phytochemicals and the same has been presented in table

Proximate composition

The nutritional composition of *Catharanthus roseus* has been tabulated in the following table.

Parameters	**Concentration in leaves**	**Reference**	**Concentration in flowers**	**Reference**
Moisture	9.64-15.72	Choudhary et al., 2014; Ekwealor et al., 2016; Esther et al., 2016; Sharma et al., 2017	10.04	Sharma et al., 2017
Fat	8.0-42.8		4.19	
Crude lipid (%)	19.68			
Ash	3.89-8.94		5.21	
Acid insoluble ash (%)	0.21			
Water soluble ash (%)	1.01			
Crude fibre (%)	1.04-2.85		1.33	
Fibre (%)	17.55			
Chlorophyll(mg/100g)	0.94		0.11	
Nitrogen	1.30			
Protein	4.74-8.08			
Crude protein	7.05-8.08			
Carbohydrate	40.25-81.21			
Dry matter	24.28-25.80			
Vitamin C (mg/100g)	0.09			
Organic content (%)	90.63			
Micronutrient				
Nutrient	Concentration in leaves (mg $100g^{-1}$)	Reference	Concentration in flowers (mg $100g^{-1)}$	Reference
Sodium	66.46-472	Sahito et al., 2001; Singh et al., 2010; Choudhary et al., 2014; Aziz et al., 2016; Ekwealor et al., 2016	38.4-231	Sahito et al., 2001; Aziz et al., 2016
Potassium	190.16-2307		176.38-2342	
Calcium	122.9-3619		140.98-605	
Magnesium	141.35-513		85.69-175	
Chromium	0.26-2.46		0.20-0.26	
Iron	4.99-104		10.56-55.90	

Zinc	2.30-5.46		4.80-7	
Aluminium	3.50-45.31		0.20-3.12	
Copper	0.37-0.775		0.20-0.536	
Lead	0.08-0.519		0.03-0.331	
Cadmium	0.02-0.363		0.01-0.184	
Manganese	3.69-18.37		2-3.27	
Nickel	0.6-0.402		0.21-0.36	
Cobalt	0.085-0.41		0.638-0.777	
Barium	0.60			
Rubidium	1.54			
Strontium	24.72			
Phosphorus	252.2			
Phytochemicals				
CONSTITUENT	Concentration in leaves	Reference	Concentration in Flowers (%)	Reference
Sterol	0.16	Esther et al., 2016; Sharma et al., 2017		Sharma et al., 2017
Flavonoid	0.0175-0.48		0.019	
Glycosides	1.76			
Alkaloid	11.84			
Saponin	0.46			
Phenol	0.0423-0.32		0.450	
Anthocyanin	0.42			

Anti-nutritional components of *C. roseus*

ANTINUTRIENTS	Concentration in leaves (mg/100g)	Reference
Hydro cyanide	0.89	Choudhary et al., 2014
Total oxalates	239	
Soluble oxalates	10.39	
Phytates	0.06	
Tannin	0.04	

Bioactive compounds present in different parts of *Catharanthus roseus*

Bioactive compounds, produced from the plant secondary metabolism, are the non-nutritional constituents of plants, and which have been found to have significant positive health effects on humans. Recently compounds like flavonoids, alkaloids and tannins, anthocyanin have been reported in various parts of *Catharanthus roseus* which are responsible for the numerous biological activities. For example, *Catharanthus roseus* contains vinca alkaloids, which is the second most and oldest plant alkaloids groups used as drug for cancer treatment (Moudi et al, 2013). Among all the bioactive compounds, alkaloid is the dominant compound present in the Vinca rosea and is responsible for numerous health benefits. Vinca alkaloids such as vincristine, vinblastine, vinorelbine were also use in the western medicines (Pan et al, 2010). It contains more than 300 alkaloids which is having antineoplastic agents to treat leukemia, hodgkine's disease (Punia et al, 2014; Rosario et al, 2015), malignant lymphomas, neuroplastoma, wilm's tumour and other cancers, also have indole alkaloid such as lochnerine, tetrahydroalstonine and vindolidine which posseses hypoglycemic effect (Barik et al, 2016). Mostly the alkaloids present are ajmalcine, vinceine, resperine, vincristine, raubasin and vinblastine (Mishra et al, 2017). Anticancer property of *Catharanthus roseus* is due to vinblastine and vincristine compounds (Mishra et al, 2017; Khanuja, 2012; Gupta and Raina, 1998). Vinblastine compound prevents germ cells, renal cancers, breast cancer and lymphoma cancers by stopping mitosis in metaphase and prohibiting microtubule formation (Mishra et al, 2017). *Catharanthus roseus* is having the phenolic compounds which includes C6C1 compounds such as 2, 3-dihydroxybenzoic acid and phenylpropanoids such as derivatives of cinnamic acid, flavonoids and anthocyanin (Barik et al, 2016). Percentage of alkaloids on dry weight basis in different parts of the *Catharanthus roseus* is root; 0.12-9.00, stem; 0.07-0.46, leaf; 0.10-1.16, fruit; 0.40, seed; 0.18, flower; 0.005, pericarp; 0.14 (Barik et al, 2016).

Mechanism of action of alkaloids present in *Catharanthus roseus* (Pandey, 2009)

Alkaloid	Mechanism	Action against
Vinblastine	Stops tubulin protein formation and stops cell division in metaphase	Lymphoma, breast and renal cancers
Vincristine	Stops tubulin protein formation and stops cell division in metaphase	Leukemia, lymphoma and cancers of breast and lungs

Flower

Flowers of *Catharanthus roseus* contain high amount of tannins, triterpenoids and alkaloids which possesses numerous biological activity such as antidiabetic and antibacterial properties (Barik et al, 2016). The flower of *Catharanthus roseus* shows high antioxidant activities 97.44% at 800µg, which is higher than standard L- ascorbic acid that shows 94% in the same concentration (Barik et al, 2016). This prevents the formation of free radicals due to the presence of volatile phenolic compounds such as glycosides, flavanols and caffeoylquinic acids (Rao et al, 2013). Anthocyanin pigment i.e. rosidin is present in this flower (Mishra et al, 2017; Das and Sharangi, 2017). *Catharanthus roseus* contains indole alkaloids in all its plant parts.

Bark

Bark of this flower contains alkaloid Alastonin that helps in regulating blood pressure (Barik et al, 2016). Bark of the root of *Catharanthus roseus* contains vincristine and vinoblastine which is used as sedative (Barik et al, 2016).

Root

In root tissues catharanthine is present which posseses antidiabetic, menorrhagia and antihypertensive properties (Barik et al, 2016). Ajmalicine is a monomeric alkaloid that cures circulatory diseases and maintains normal cerebral blood flow by increasing the blood flow in the brain and pheripheral parts of the body (Barik et al, 2016). Roots also contain quinones which are antibacterial in function (Barik et al, 2016). Flavonoids, triterpenoid, tannins, saponin, coumarin, quinones and phenolic compounds are also present which possess antioxidant activity

which prevents enzymatic and non-enzymatic oxidation (Barik et al, 2016). *Catharanthus roseus* has the highest oxygen radical absorbance capacity (ORAC) (Barik et al, 2016). Roots contains antihypersensitive alkaloids such as serpentine, ajmalicine, reserpine (Rao et al, 2013).

Leaves

Leaves contain good amount of alkaloids and polyphenols. Polyphenols exhibit antioxidant properties. *C.roseus* contains 130 alkaloids of indole group from which 25 are dimeric in nature and leaves produce two important commercially cytotoxic dimeric alkaloids which is vinblastine and vincristine (Barik et al, 2016). Vincristine and vinblastine are helpful in the treatment of leukemia and lymphoma. The ethanolic extract of *Catharanthus roseus* leaves, estimated by HPLC (High pressure liquid chromatography) method shows the presence of natural antioxidants such as quercetin and rutin (Rao et al, 2013). The leaves of *Catharanthus roseus* helps in reducing the blood glucose level and hence in curing diabetes (Barik et al, 2016; Sen et al, 2008)

Major constituents in different parts of *Catharanthus roseus*

Plant part	Major constituents	Reference
Flower	Saponin, carbohydrate, alkaloid, tannin, steroid	Jarald et al., 2006
Leaf	Alkaloid, vindoline, vinblastine, carbohydrate, vincristine, tannin, saponin, avonoid, chlorogenic acid, steroid, triterpenoid, loganic acid, vincristine, secologenin	Jarald et al., 2006; Siddiqui et al., 2010; Guimaraes et al., 2012
Stem	Alkaloid, carbohydrate, avonoid, steroid, tannin	Jarald et al., 2006
whole plant	Vinblastine, catharanthine, vincristine, vindoline, monoterpenoid, 7-O-methylated anthocyanin, glycoside, steroid, phenolic, avonoid,	Kulkarni et al., 1999; Williams et al., 2004; Jaleel et al., 2008; Siddiqui et al., 2010; Guimaraes et al., 2012
Root	Serpentine, saponin, carbohydrate, alkaloid, tannin, alkaloid, ajmalicine, steroid, triterpenoid	Kulkarni et al., 1999; Jarald et al., 2006

Alkaloids present in different parts of *Catharanthus roseus*

Plant part	Alkaloids	Properties	Reference
Root	Ajmalicine	Antihypertensive and controls cardiovascular diseases	Jaleel et al., 2006; Ferreres et al., 2008; Kotakadi et al., 2013 Punia et al, 2014; Kumar et al, 2015; Barik et al 2016
	Catharanthine	Antidiabetic	
		Leukaemia, anti-diabetic, Anti-hypertensive, relieves menorrhagia	
	Raubasin	Pain relieving	
	Reserpine	Tranquilizer	
	Serpentine	Antihypertensive and controls cardiovascular diseases	
Leaf	Vinblastine	Anticancer property	
	Vincristine	Anticancer property, Helps to treat leukemia in children, choriocarcinoma, and Hodgkin's disease	
	Vindoline	Anticancer property Antidiabetic property Anti-ulcer property	
	Vincamine	Neuroprotective, Cerebro-vasodilatory, Anti-ulcer property	
Bark	Alastonin	Regulate blood pressure	
Stem	Vincristine Vinblastine	Antitumor property	

Medicinal properties of different parts of *Catharanthus roseus*

Bioactivities	Plant part used	Method	Remarks	Reference
Antimicrobial	Leaves	Ethanolic extract	Ethanolic extract of *Catharanthus roseus* showed Antiplasmodial activity	Kumar et al., 2012
			Ethanolic extract of *Catharanthus roseus* leaves at concentrations 50, 75, 100% showed inhibited the growth of *Macrophomia phaseolina* and *Sclerotium rolfsii*	Wadikar et al.,2010
			Showed antimicrobial activity against *Psedomonas aeuroginosa* and *Staphylococcus aureus*	Nayak et al., 2006
		Silver nano particles of dried leaves	Showed antimicrobial activity against *Bacillus species, Lactobacillus, Staphylococcus aureus, Pseudomonas flucorescens*	Kotakadi et al., 2013
		Methanolic extract (Concentration 12.5mg and 25mg)	*Klebsiella pneumonia, Salmonella paratyphi*	JayaKumar et al, 2010
		Ethanolic extract	Showed antimicrobial activity against *Escherichia coli, Staphylococcus aureus, salmonella typii, streptococcus pyrogens, Pseudomonas aeruginosa, Serratia marcescens, Bacillus subtilis*	Ramya, 2008
		Methanolic extract	Showed antimicrobial activity against *Psedomonas aeuroginosa, Serratia marcescens, salmonella typii, Staphylococcus aureus, streptococcus pyrogens, , Bacillus subtilis*	
		Ethanolic extract (dried powder)	Showed antibacterial activity against *Escherichia coli, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia*	Goyal et al., 2008
		Ethanolic extract	Showed antibacterial against *Escherichia coli,*	

		(fresh sample)	*Salmonella paratyphi, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus*	
		Methanolic extract (dried powder)	Showed antibacterial against Salmonella paratyphi, *Klebsiella pneumonia, Staphylococcus aureus*	
		Methanolic extract (Fresh sample)	Showed antibacterial against Salmonella paratyphi, *Klebsiella pneumonia, Staphylococcus aureus*	
		Hot water extract	Showed antibacterial against Salmonella paratyphi, *Klebsiella pneumonia, Staphylococcus aureus*	
		Ethanolic extract	Shows antimicrobial activity against *Escherichia coli, Klebsiella oxytoca, Proteus mirabilis, Salmonella paratyphi*	Balaabirami and Patharajan., 2012
	Stem	Ethanolic extract (dried powder)	Showed antibacterial activity against *Escherichia coli, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus*	Goyal et al., 2008
		Methanolic extract (dried powder)	Showed antibacterial activity against *Staphylococcus aureus, Staphylococcus aureus*	
		Ethanolic extract	Showed antimicrobial activity against *Bacillus subtilis*	Ramya, 2008
		Methanolic extract	Showed antimicrobial activity against *Psedomonas aeuroginosa, salmonella typii, Staphylococcus aureus, Serratia marcescens, Bacillus subtilis, streptococcus pyrogens*	
		Methanolic extract (concentration 12.5mg) (Concentration	*Salmonella paratyphi* *Escherichia coli, Staphylococcus aureus, Salmonella paratyphi, Salmonella typhi*	Jayakumar., 2010

		25mg)		
	Root	Ethanolic extract	Showed antimicrobial activity against *Bacillus subtilis*	Ramya, 2008
		Methanolic extract	Showed antimicrobial activity against *Bacillus cereus, Bacillus subtilis, salmonella typii, Staphylococcus aureus, streptococcus pyrogens*	
		Ethanolic extract (dried powder)	Showed antibacterial activity against *Escherichia coli, Salmonella paratyphi, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Bacillus cereus*	Goyal et al., 2008
		Ethanolic extract (fresh powder)	Showed antibacterial activity against *Escherichia coli, Salmonella paratyphi, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis*	
		Methanolic extract (dried powder)	Showed antibacterial activity against *Salmonella paratyphi, Klebsiella pneumonia, Staphylococcus aureus*	
		Methanolic extract (fresh sample)	Showed antibacterial activity against *Escherichia coli, Salmonella paratyphi, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis*	
		Methanolic extract (concentration 12.5mg) (concentration 25mg)	*Klebsiella pneumonia, Staphylococcus aureus* *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Salmonella paratyphi, Shigella sonnei, salmonella typii*	JayaKumar et al, 2010
	flower	Ethanolic extract (dried powder)	Antimicrobial against *Escherichia coli, Salmonella paratyphi, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus*	Goyal et al., 2008
		Ethanolic extract	Antibacterial activity against *Bacillus cereus*	Ramya, 2008

		Methanolic extract	Antibacterial activity against *Staphylococcus aureus, Bacillus cereus, salmonella typii, Streptococcus pyrogens*	
		Methanolic extract (concentration 12.5mg) (Concentration 25mg)	*Escherichia coli, Salmonella paratyphi, Shigella sonnei* *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Shigella sonnei, Salmonella paratyphi, Salmonella typhi*	JayaKumar et al, 2010
	Whole plant	Aqueous extract	Antimicrobial against *Chromobacterium, Escherichia coli, Enterobacter faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella paratyphi, Salmonella typhi*	Srinivasan et al., 2001
	Seed	Methanolic extract (Concentration 25mg)	*Escherichia coli, Bacillus cereus*	JayaKumar et al, 2010
Antifungal	Leaves	Ethanolic extract	*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium species*	Balaabirami and Patharajan., 2012
Antidiabetic	Whole plant	Alloxan induced diabetic rats (150mg/kg)	Methanolic extract of whole plant of *Catharanthus roseus* showed antihyper glycemic activity at a dose of 500mg/kg for days Regeneration of β cells of pancreas in diabetic rats	Ahmed et al., 2010
	Leaves	Alloxan induced diabetic rats (100mg/kg)	Aqueous extract of leaves given at a dose of 0.5, 0.75, 1.0ml/kg body weight showed reduction in blood glucose level in alloxan induced diabetic rats by increasing the secretion of insulin from	Nammi et al., 2003

			β cells of langerhans through extrapancreatic mechanism.	
		Streptozotocin induced diabetic rats (75mg/kg)	There is lowering of blood glucose level in streptozotocin induced diabetic rats when they are treated with crude aqueous extract at a dose of 1g/kg for 21days	Singh et al., 2001
		Streptozotocin induced male wistar rats (55mg/kg body weight)	There is reduction in plasma glucose and increase in plasma insulin in streptozotocin induced male wistar rats when treated with suspension of *Catharanthus roseus* leaf powder at dose of 100mg/kg/body weight for 60 days	Rasineni et al., 2010
		Alloxan induced diabetic rats (110mg/kg)	Ethanolic extract of catharanthus leaves when given at a dose of 300mg/kg reduces the blood glucose level of diabetic rats.	Akhtar et al., 2007
		Alloxan induced diabetic rats (110mg/kg)	Methanolic extract of *Catharanthus roseus* leaves (250 mg/kg for 7 days) and extract- drug (metformin) (100mg/kg and 250 mg/kg for 7 days) combination showed lowering of blood glucose level in diabetic rats	Ohadoma et al., 2011
		Alloxan induced diabetic rats (80mg/kg)	Crude dichloromethane:methanol (1:1) leaves extract of *Catharanthus roseus* given at a dose of 500mg/kg for 20 days showed decrease in blood glucose level	Jayanthi et al., 2010
		Streptozotocin induced inbred Swiss albino rats	Hydroalcoholic extract of *Catharanthus roseus* leaves given at a dose of 50mg/kg decreases the blood glucose level in normal and diabetic model rats	Chattopadhyay et al., 1999
		Alloxan induced rabbits	Leaf juice of *Catharanthus roseus* lowers the blood glucose level in alloxan induced rabits	Satyanarayana et al., 2003
		Alloxan induced male albino rats (150mg/kg)	Aqueous extract of *Catharanthus roseus* given at a dose of 4mg/kg for 7 days lowers blood glucose level in diabetic rats	Ghosh et al., 2001
		Streptozotcin	Ethyl acetate extract of catharanthus leaves	Islam et al., 2009

		induced Long Evan females (45mg/kg)	given at a dose of 150mg/kg showed reduction in blood glucose level in diabetic rats	
	Leaves and twigs	Streptozotocin induced diabetic rats (75mg/kg)	Dichloromethane:methanol (1:1) extract of leaves and twigs of *Catharanthus roseus* given at a dose of 500mg/kg showed hyperglycemic activity in streptozotocin induced diabetic rats	Singh et al., 2001
	Flowers	Alloxan induced male albino rats (150mg/kg)	Aqueous extract of *Catharanthus roseus* flowers 10ml/kg for 7 days decreases the blood glucose level in diabetic rats	Ghosh et al., 2001
Anticancer	Whole plant	Antiproliferative active assay	Methanolic extract of *Catharanthus roseus* showed antiproliferative activity against metastatic-1080 fibrosarcoma cells	Ueda et al., 2002
		Chlorioallantoic membrane assay (CAM) and In vitro bovine aortic endothelial cells ($BAEC_S$)	Boiling extract of *Catharanthus roseus* posess activity in both Chick embryo chlorioallantoic membrane (CAM) and Bovine aortic endothelial cells culture model ($BAEC_S$)	Wang at al., 2004
	Root and Aerial parts	MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay)	Methanolic extract of roots and aerial parts of *Catharanthus roseus* reduced the proliferation of human ductal breast epithelial tumour cell lines (T47D) with a mean inhibition concentration (IC_{50}) of 2.8%.	Widowati et al., 2013
		MTT cell proliferation assay	Methanol extract of catharanthus leaves showed dose independent cytotoxic activity against HCT-116 colorectal carcinoma cell line. Methanol, n-hexane and chloroform fractions showed dose independent cytotoxic activity against HCT-116 colorectal carcinoma cell line	Siddiqui et al., 2010

			Pure alkaloids Catharanthine and Vindoline extracted from the leaves of Catharanthus leaves showed dose dependent cytotoxic activity against HCT-116 colorectal carcinoma cell line	
	Leaves and stem	Vincristine, vinblastine	Prevents breast cancer, soft tissues sarcomas, Hodgkin's disease, leukemia in children	Ferreres et al., 2008
Wound healing	Flowers	Incision wound Sprague Dawley rats	Ethanolic extract of *Catharanthus roseus* flowers given at a dose of 100mg/kg/day increased the wound healing capacity in incision wound Sprague Dawley rats	Nayak et al., 2006
	Leaves	Excision wound Male Sprague Dawley rats	Methanolic extract of *Catharanthus roseus* leaves showed wound healing activity in Male Sprague Dawley rats when given at a dose of 100mg/kg/day	Nayak et al., 2007
Antioxidant	Leaves	DPPH assay	Crude ethanolic extract of *Catharanthus roseus* leaves showed antioxidant activity of 25.58% inhibition.	Fawole et al., 2013
		Ferric reducing antioxidant power assay (FRAP)	Crude ethanolic extract of *Catharanthus roseus* leaves showed antioxidant activity of 151.03(µmol Fe(II)/g dry weight of the extract)	
		Total phenol content assay (TPC)	Crude aqueous extract of *Catharanthus roseus* showed antioxidant activity of 140.81 (mg GAE/ 100g dry weight of extract)	
		DPPH assay	Methanolic extract *Catharanthus roseus* leaves showed antioxidant potential of 83.72% at concentration 800µg	Jayakumar et al., 2010
		TPC assay	Leaves of *Catharanthus roseus* have 422.56mg/100g of phenolic content which shows it is having high antioxidant activity	Sharma et al., 2017
		TFC asaay	Leaves of catharathus roseus have 17.50mg/100g of phenolic content which shows it is having high antioxidant activity	
	Stem	DPPH assay	Methanolic extract *Catharanthus roseus* stem	Jayakumar et al., 2010

			showed antioxidant potential of 93.75% at concentration 800µg	
	Root	DPPH assay	Methanolic extract *Catharanthus roseus* root showed antioxidant potential of 93.84% at concentration 800µg	
		Hydroxyl radical scavenging activity	Ethanolic extract of *Catharanthus roseus* showed good amount of radical scavenging activity in all the radical scavenging assays.	Bhutkar and Bhise., 2011
		Superoxide radical scavenging activity		
		DPPH assay		
		Nitric oxide radical inhibition method		
	Flower	DPPH assay	Methanolic extract *Catharanthus roseus* flower showed antioxidant potential of 97.44% at concentration 800µg	Jayakumar et al., 2010
		TPC assay	Flowers of catharathus roseus have 450.20mg/100g of phenolic content which shows it is having high antioxidant activity.	Sharma et al., 2017
		TFC assay	Flowers of *Catharanthus roseus* have 19.00mg/100g of flavonoids which shows it is having good amount of antioxidant property	
	Seed	DPPH assay	Methanolic extract *Catharanthus roseus* seed showed antioxidant potential of 80.28% at concentration 800µg	Jayakumar et al., 2010
	Aerial and roots	DPPH assay	Methanolic extract of roots and aerial parts of *Catharanthus roseus* showed antioxidant activity of 71.87%	Widowati et al., 2013
	Shoots	DPPH assay	100% Methanolic extract and 100% ethylacetate fraction of *Catharanthus roseus* showed significant antioxidant activity	Rasool et al., 2011
		Total phenol content assay (TPC)	100% Methanolic extract of *Catharanthus roseus* showed significant antioxidant activity 8.5g/100g	

		Total Flavonoid content assay (TFC)	100% Methanolic extract of *Catharanthus roseus* showed significant antioxidant activity of 19.8g/100g	
Hypolipidemic	Leaves	UV Spectrophotometer	Leaf juice of *Catharanthus roseus* reduces serum total cholesterol, total triglycerides, LDL-cholesterol, and VLDL-cholesterol in Albino wistar rats	Antia et al., 2005
		UV spectrophotometer	Ethanolic extract of catharanthus leaves given at dose of 150mg/g to normal and alloxan induced diabetic rats lowers their and serum glycerides	Akhtar et al., 2007
		UV spectrophotometer	Ethyl acetate extract of catharanthus leaves given at a dose of 150mg/kg reduced serum triglyceride level in streptozotocin induced diabetic rats	Islam et al., 2009
		Estimation method (Friedewald et al., 1972)	Leaf juice of *Catharanthus roseus* lowers the total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, HDL-cholesterol	Patel et al., 2011
Antiulcer	Leaves	Experimentally induced gastric damage in rats (96% ethanol)	Vinpocetine alkaloid present in the catharanthus leaves showed antiulcer activity against experimentally induced gastric damage in rats	Nosalova et al., 1993
		Vincamine and Vindoline	They possess antiulcer property	Sain et al, 2013
Anthelminthic	Whole plant	Earthworms (*Pheretima posthuma)*	Ethanolic extract given at a dose of 200mg/ml showed anthelminthic activity with death time of 46.33 minute Ethanolic extract showed paralysis effect in 6.67 minute	Agarwal et al., 2011
Antidiarrheal	Aerial parts	Castor induced wistar rats	Ethanolic extract given at a dose of 500mg/kg showed antidiarrheal activity in castor induced wistar rats	Rajput et al., 2011
Hypotensive	Root	Alstonine, Ajmalicine,	Helps in lowering blood pressure	Kotakadi et al., 2013

		Serpentine, Reserpine		
Transquilizer	Root	Reserpine	It helps in reducing pain	Jaleel et al., 2006
Sedative	Root	Raubasin	It helps in lowering down anxiety, excitement	
Memory enhancement	Aerial part	Vincamine , Vinpocetine	Vincamine and Vinpocetine improves the brain function by enhancing the ability to utilise glucose and oxygen.	Rathee et al., 2008

Therapeutic importance of *C. roseus*

Traditionally people utilize this crop because of its numerous health benefits such as antidiabetic, anticancer, antimicrobial, antioxidant, antihelmintic, antidiarrheal, antiulcer, antihypertensive, phytoremediation, antimutagenic, memory enhancement activity, wound healing property, blood cleanser, antihypercholesterolemic, disinfectants, tranquilizing and sedative actions throughout the world (Ramesh and Subramani, 2015; Kumar 2014), (Balaji et al, 2014; Kumar 2014; Vaidya and Devasagayam, 2007, Kumar et al, 2014).

The different parts of plant have been reported to have wide application in various parts of the world, where its extract is being used for curing rheumatism, menorrhagia, indigestion, dyspsia, diabetes, eye problems, cancer, asthma, tuberculosis, sleeping disorders, hypertension, chest ailments, sore throat etc.

Traditional uses of *Catharanthus roseus* in different parts of the world

Plant part	Traditional uses	Reference
Plant leaves	• Traditionally the extract of leaves utilized for treatment of wasp sting. • Cures rheumatism, menorrhagia, diabetes, diarrhoea, indigestion, dyspepsia, haemorrhage, scurvy. • Juice extract of plant leaves was used to cure indigestion and dyspepsia problem. • It was also used as mouthwash for toothache, cleaning and healing of chronic wounds.	Sain et al, 2013; Ndip et al, 2013; Aruna et al, 2015; Aziz et al, 2016; Das and Sharangi, 2017
Flower extract	Used for eye washing of infants and cure irritation	
Root	• Traditionally used for laxative effect • concentrated extract of root is used for dysentery, intestinal parasitism • crude extract of roots have anticancer property	
Whole plant	• Concentrated plant juice to cure diabetes. • Concentrated extract of *Catharanthus roseus* is used to cure asthma, flatulence and tuberculosis. • *Catharanthus roseus* was used to treat diabetes, cancer, insomnia and hypertension. • Extract of plant was used to cure sore throats, chest ailments, laryngnitis by gargling. • Concentrated juice of young plant leaves to cure stomach cramps • Crude extract of leaves and roots have anticancer property. • People use boiled plant extract to stop bleeding.	

Chapter5: PROPOSED RESEARCH OBJECTIVES

The mandate of the present study is as under –

(1) To standardize the drying technology for Vinca rosea.

(2) To check the suitability of different extraction methods (eco-friendly and microwave assisted) for the extraction of phytochemicals from the Vinca rosea.

(3) Preparation and evaluation of Vinca rosea enriched fruit based beverages.

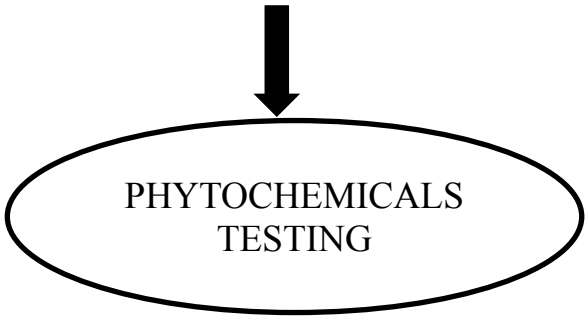
Chapter 6: PROPOSED RESEARCH METHODOLOGY

EXPERIMENT 1 (a) - Drying of different parts of Vinca rosea at different temperatures and determination of its phytochemicals retention.

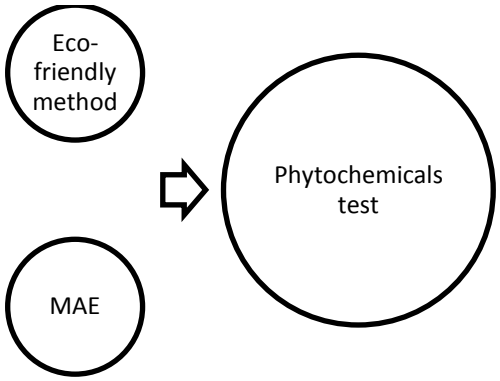
Plant parts	**Drying temperature**
Leaf	45°C
Stem	55°C
Flower	65°C

No. of treatments = 3×3=9

Number of replication = 3

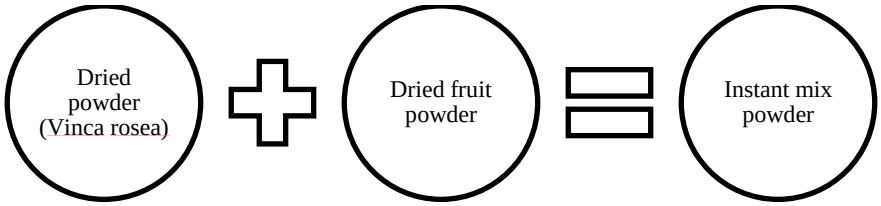


EXPERIMENT 1 (b) – The plant part with maximum phytonutrient retention will undergo MAE (microwave assisted extraction) and eco- friendly extraction.

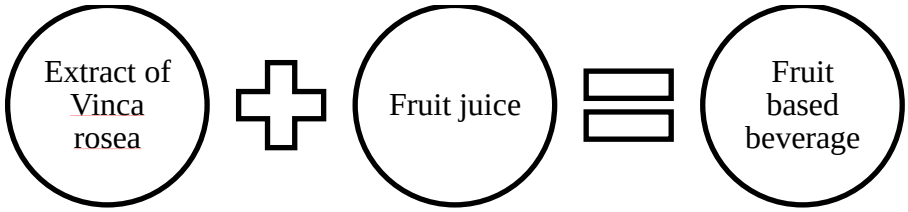


EXPERIMENT NO. 2 – Development of Vinca rosea enriched fruit based beverages and instant beverage mix powder.

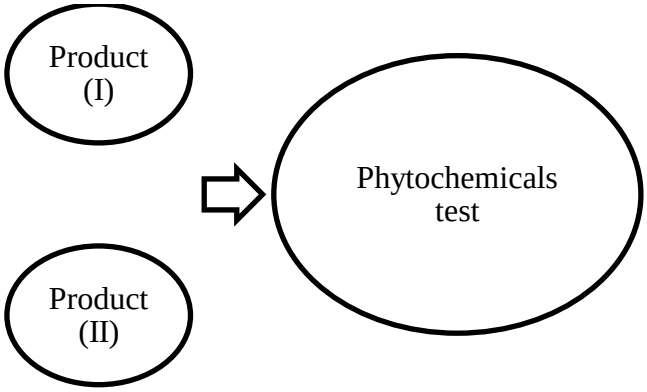
-From experiment 1(a)



-From experiment 1(b)



- Phytochemical testing



EXPERIMENT NO. 3 – Storage studies of the developed beverages by using different packaging materials and at different storage conditions.

Storage conditions	Storage durations (days)
Ambient	0
Refrigerated	30
	60
	90

No. of treatments = 2×4=8

Number of replication = 3

EXPERIMENT NO. 4- Consumer studies of developed products.

Consumer studies for the acceptance and rejection of the product will be carried out using the hedonic scale.

6.1 Chemical composition

6.1.1 Protein content	AOAC 2000
6.1.2 Fat content	Ranganna 2016
6.1.3 Crude fibre content	AOAC 2000
6.1.4 Moisture content	AOAC 2000
6.1.5 Ash content	AOAC 2000
6.1.6 Sugars	AOAC 2000
6.1.7 Dietary fibre content	AOAC 2000

6.2 Phytonutrient composition

6.2.1 Flavonoids	AOAC 2000
6.2.2 Tannins	AOAC 2000
6.2.3 Phytic acid	AOAC 2000
6.2.4 DPPH assay	AOAC 2000
6.2.5 Metal chelation	AOAC 2000
6.2.6 Ascorbic acid	AOAC 2004
6.2.7 Phenols	AOAC 2000
6.2.8 FRAP	AOAC 2000
6.2.9 Chlorophyll	AOAC 2000
6.3 Antimicrobial activity	AOAC 2000
6.3.1 Antidiabetic activity	AOAC 2000
6.3.2 FTIR	AOAC 2000

6.4 Organoleptic evaluation

Chapter 7: EXPECTED RESEARCH OUTCOME

The dried flowers of *Vinca rosea* will be rich in anti-oxidant, anti-microbial, anti-diabetic and antidiarrheal properties. The developed food product will help to boost the immunity, thus providing health benefits to the consumers. The product will serve as an economic functional food for diabetic patients. The high amount of antioxidants, amino acids, proteins and other vital nutrients in the product will be suitable and beneficial for all economic strata. Not only the diabetics but people suffering from majority of life threatening ailments will benefit from this product. Our main aim is to standardize the drying technique in which the dried flowers will have the retention of maximum amount of nutrients in it.

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