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Studying the Crude Extract and Biological Activity of *Benincasahispida*: A Review

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ABSTRACT: *Benicasahispida* is a common vegetable found in subtropical and tropical Asia belonging to the family of *Cucurbitaceae* and considered to be highly beneficial due to its Phytochemistry. It has been used as food and medicine for a thousand of years in Orient. Both the mature and young vegetable can be consumed but only the matured one has seeds in it. All parts of *Benincasa hispida* have medicinal property. It shows antimicrobial, antioxidant, anticancer, antidiabetic activity, antiinflammatory and analgesic activity. *Benincasa hispida* is found to inhibit as wide range of gram negative and gram positive bacteria that are human pathogens.

KEYWORDS: *Benincasahispida*, food and medicine, Antioxidant, Anticancer, Antidiabetic activity

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I. INTRODUCTION

Fruit and vegetable has been a part of human diet for a long period of time. Some of them has been known to cure certain illness and was termed as medicinal plant. Fruit and vegetable from such medicinal plant are consumed as such or as juice or in dried form or as an extract in order to cure certain illness or disease. Even common fruits and vegetable which is used in our regular day to day life also has certain health benefits when consumed in appropriate quantity in the form of balanced diet. One such vegetable is *Benincasa hispida* it is a vegetable that has been commonly used in cooking in various parts of Asia. Fig. 1 shows the photograph of *Benincasahispida* .*Benincasahispida* is a vegetable widely found in subtropical and tropical Asia. It is also known as ash gourd, wax gourd, white gourd, white gourd, etc. It is a drought resistant but prefers moist soil for growth. All parts of *Benincasa hispida* is used in medicine and as well as food for a thousand of years in Orient. Table 1 depicts the taxonomy classification of *Benincasahispida.* Both the mature and young vegetable can be consumed but only the matured one has seeds in it. It can be cooked as curry or can be candied. It is profoundly used in Indian cooking and traditional purposes. Seeds are known to contain oil and protein.

Figure 1: A picture of *Benincasa hispida* (Ash gourd).

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Table 1: Taxonomy of *Benincasahispida*.

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Cucurbitales
Family	Cucurbitaceae
Subfamily	Cucurbitoideae
Tribe	Benincaseae
Genus	*BenincasaSavi*
Species	*B. hispida*

II. PHYTOCHEMISTRY

Sheemole*et al*., have reported the phytochemicals present in *Benincasa hispida* pulp using Liquid Chromatography Mass Spectrophotometric Analysis (LC-MS). The phytochemical analysis using methanol and petroleum ether extract revealed the presence of the following primary metabolites - carbohydrate, fatty acids, organic acids, amino acids as well as secondary metabolites - phenol, Flavonoids, phenolic acids, Alkaloids, terpenoids, Coumarin, sterols. The applying computerized system PASS it is revealed to contain several pharmacological activities like antivirus, anti-carcinogenic, anti-seborrheic, free radical scavenger, hypercholesterolemic, cardio protectant, sickle cell anemia treatment, chemo protective, Lipid metabolism regulator etc.

VedhaBalakumar*et al*., have demonstrated that the bioactive compound profile of *Benincasa hispida* fruit using GC-MS Analysis by successive extraction. Fruit extracts was prepared with chloroform, methanol and aqueous based on their polarity. The GC-MS Analysis revealed that in Methanolic extract 22 peaks of components were identified. In chloroform extract 16 compounds were identified. In aqueous extract13 compounds were identified (Table 2).

Table 2: The major compounds of different extracts.

Methanolic extract	Chloroform extract	Aqueous extract
α1-Sitosterol (97.24%)	4HPyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (100 %)	Methanecarboxylic acid (100%)
Lupan-3-ol (56..83 %)	Methanecarboxylic acid (34.49 %)	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (21.64%)
Cucurbitacin B, dihydro-(25.29 %)	α-Pyrrolidone, 5-[3-hydroxybutyl]-(10.01%)	Cyclotrisiloxane, hexamethyl (11.36%)
cis-13-Octadecenoic acid (20.56 %)	Ethanoic acid (6.88%)	2-Hydroxy-gammabutyrolactone(8.00%).
3-Hydroxy-3-methyl-2- butanone oxime 5.16%)		
1,2- Cyclopentanedione (4.16%)		

However, only methanolic and chloroform extracts showed distinct class of compounds compared to aqueous extract.RekhaJadon*et al*., have performed tests to confirm the presence of the active chemical constituents (alkaloids, glycosides, terpenoids, flavonoids, reducing sugar, saponins and tannins) (Table 3).

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Table 3: Indicates the presence or absence of the phytochemical.

Tests	Presence/Absence
Alkaloids	-
Glycosides	+
Terpenoids	+
Flavonoids	-
Reducing sugar	-
Saponins	-
Tannins	-

Gill *et al*.,have performed phytochemical tests in methanolic extract of seed of *B.hispida* using standard procedure (Table 4).

Table 4.Indicates the presence or absence of the phytochemical
+ Presence, - Absence

Test	Reagents	Results
Alkaloids	Dragendroff's reagent, Hagers reagent, Mayer's reagent, Wagners reagent.	-
Flavonoids	Concentrated nitric acid	-
Protein and amino acid	Ninhydrin reagent	+
Triterpenoids	Liebermann-Burchard's test	+
Saponin		-
Carbohydrates	Molish test	+
Phytosterols	Liebermann test	+
Tannins	Ferric cholride	+

III. ANTIMICROBIAL ACTIVITY

Sunayana Sharma *et al*., have reported the antibacterial activity of Hispidalin isolated from the seeds of *Benincasa hispida*. The antibacterial activity of peptide was determined by paper disc fusion method. The test was performed against *E.coli, S.enterica, P.aeruginosa, B.cereus* and *S.aureus*. Ciprofloxacin was used as positive control and water as negative control. In this test Hispidalin showed maximum zone of inhibition against *S.enterica* and minimum zone of inhibition against *P.aeruginosa*. This concluded that *B.hispida* seeds showantibacterial activity against human pathogens (Table 5).

Table 5.Indicates the presence or absence of zone of inhibition.

Microorganism	Zone of inhibition
E.coli	+
P.aeruginosa	+
B.cereus	-
S.enterica	+
S.aureus	+

+ Presence, - Absence

In an another study, Wafaa E. Soliman *et al*., have studied the antibacterial activity of AgNPs synthesized from the peel extract of *B.hispida* by disk diffusion. The antibacterial activity was performed against pathogenic Gram positive and Gram negative bacterial strains (*S.aureus, M.luteuns, E.coli* and *K.pneumoniae*). AgNPs showed maximum activity against *E.coli (*Gram negative) and (*M.luteuns*) compared to other pathogenic bacterial strains (Table 6).

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Table 6.Indicates the presence or absence of zone of inhibition

Microorganism	Zone of inhibition
S.aureus	+
M.luteuns	+
E.coli	+
K.pneumoniae	+

+ Presence, - Absence

Natarajan*et al*., have reported the antibacterial activity of Methanolic extract of *B.hispida* fruit against *S.aureus, S.epidermidis, B.subtilis, E.coli, P.aeruginosa* and *K.pneumoniae* by cup - plate method (Table 7). Ciprofloxacin solution was used as standard solution. Methanolic extract of *B.hispida* fruit showed no antibacterial activity against any of the bacterial strain both in higher concentration as well as in lower concentration.

Table 7.Indicates the presence or absence of zone of inhibition.

Microorganism	Zone of inhibition
S.aureus	-
S.epidermidis	-
B.subtilis	-
E.coli	-
P.aeruginosa	-
K.pneumoniae	-

+ Presence, - Absence

RekhaJadon*et al*., have reported that the pure juice and diluted juice extract of *B.hispida* was ineffective against *E.coli, B.subtilis*and*S.aureus* bacterial strains (Table 8).

Table 8.Indicates the presence or absence of zone of inhibition.

Microorganism	Pure juice	Diluted juice (1:10)
E.coli	-	-
B.subtilis	-	-
S.aureus	-	-

+ Presence, - Absence

Rajesh Kumar Shah *et al*., have performed antimicrobial activity of leaf and seed extracts of *B.hispida*. The extract wasprepared using different solvent (Methanol, Ethanol, Chloroform and Aqueous). Ampicillin and Cefotaxime were used as positive control and DMSO was used as negative control. The extracts were tested against *S.aureus, E.coli, P.aeruginosa, Proteus mirabilis* and *B.cereus* by agar well diffusion method. The Methanolic and Ethanolic seed extracts were more effective against bacterial strains than chloroform and aqueous extracts. . Ethanolic seed extract produced maximum zone of inhibition in *B.cereus.*

A. Antimicrobial activity of seed extract

Table 9: Indicates the presence or absence of zone of inhibition

Microorganism	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract
B.cereus	+	+	+	-
S.aureus	+	+	-	-
E.coli	+	+	+	+
P.aeruginosa	+	+	+	-
Proteus mirabilis	+	+	-	-

+ Presence, - Absence

Similarly the Methanolic and Ethanolic leaf extracts were more effective against bacterial strains. Whereas, Chloroform and Aqueous extract were ineffective.

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Antimicrobial activity from leaf extract

Table 10: Indicates the presence or absence of zone of inhibition

Microorganism	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract
B.cereus	+	+	-	-
S.aureus	+	+	-	-
E.coli	+	+	-	-
P.aeruginosa	+	+	-	-
Proteus mirabilis	+	+	-	-

+ Presence, - Absence

Rajesh Kumar Sharma *et al*., have performed antibacterial activity of seed extracts of *B.hispida.* The extract was prepared using different solvent (acetone, chloroform and water). The extracts were tested against *E.coli* (Gram negative) and *S.aureus* (Gram positive) by using disk plate diffusion method in different dose concentration ranging from 300-500 mg/ml. All the extract showed zone of inhibition in both *E.coli*and*S.aureus*.

Table 11: Indicates the presence or absence of zone of inhibition

Treatment	zone of inhibition against *E.coli*	zone of inhibition against *S.aureus*
Acetone extract 300 mg/ml	+	+
Acetone extract 400 mg/ml	+	+
Acetone extract 500 mg/ml	+	+
Chloroform extract 300 mg/ml	+	+
Chloroform extract 400 mg/ml	+	+
Chloroform extract 500 mg/ml	+	+
Aqueous extract 300 mg/ml	+	+
Aqueous extract 400 mg/ml	+	+
Aqueous extract 500 mg/ml	+	+

+ Presence, - Absence

The Acetone extract showed maximum inhibition in both *E.coli* and *S.aureus*. The Chloroform extract showed minimum inhibition in both *E.coli*and*S.aureus*.

IV.ANTIFUNGAL ACTIVITY

Sunayana Sharma *et al*., have described the antifungal activity of Hispidalin isolated from the seeds of *Benincasa hispida*. The antifungal activity was studied against five pathogenic fungi *A.flavus, F.solani, P.chrysogenum, C.geniculata* and *C.gloeosporioides* by paper disc fusion method. *Griseofulvin* was used as positive control. Hispidalin showed maximum growth of inhibition in *A.flavus* and minimum growth of inhibition in *C.geniculata*

Table 12. Indicates the presence or absence of zone of inhibition

Microorganism	zone of inhibition
A.flavus	+
F.solani	+
C.geniculata	+
C.gloeosporioides	-
P.chrysogenum	+

+ Presence, - Absence

Natarajan*et al*., have performed the antifungal activity of methanolic extract of *B.hispida* fruit against *Candida albicans* and *Aspergillus niger* by cup - plate method. Ketaconazol solution was used as standard solution. Methanolic extract produced zone of inhibition against *Candida albicans*in higher concentration and produced no zone of inhibition

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against *Aspergillus niger* in both higher concentration as well as in lower concentration. Thus Methanolic extract of *B.hispida* fruit can be used in the treatment of Candidiasis.

Table 13. Indicates the presence or absence of zone of inhibition

Microorganism	Zone of inhibition
Candida albicans	+
Aspergillus niger	-

+ Presence, - Absence

RekhaJadon*et al*., have reported that the pure juice and diluted juice extract of *B.hispida* was ineffective against *Aspergillus niger*and *P.chrysogenum* fungal strains.

Table 14. Indicates the presence or absence of zone of inhibition

Microorganism	Pure juice	Diluted juice (1:10)
Aspergillus niger	-	-
P.chrysogenum	-	-

+ Presence,- Absence

Rajesh Kumar Shah *et al*., have performed the antimicrobial activity of leaf and seed extracts of *B.hispida*. Ampicillin and Cefotaxime were used as positive control and DMSO was used as negative control. The extracts were prepared using different solvent (Methanol, Ethanol, Chloroform and Aqueous). When the extracts were tested against *Aspergillus niger* by agar well diffusion method Methanolic leaf extract produced maximum zone of inhibition against *Aspergillus niger*.

Antimicrobial activity from seed extract

Table 15. Indicates the presence or absence of zone of inhibition

Microorganism	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract
Aspergillus niger	+	+	-	+

+ Presence, - Absence

Antimicrobial activity from leaf extract

Table 16: Indicates the presence or absence of zone of inhibition

Microorganism	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract
Aspergillus niger	+	+	-	-

+ Presence, - Absence

V. ANTIDIABETIC ACTIVITY

Raju N Patil *et al*., have tested the activity of *B.hispida* on allogen induced diabetic rats. The dried powder of *B.hispida* was extracted by chloroform. Tolbutamide was used as standard antidiabetic. The study showed that the diabetic rat treated with *B.hispida* at dose 250 and 500/mg kg body weight showed significantly low blood glucose level.

VI. ANTIOXIDANT ACTIVITY

DPPH Radical savengingactivity

NadiraBinteSamad*et al*., haveperformed DDPH Radical savenging activity in *B.hispida* seed with BHT (butylated hydroxytoluene) as reference. The stock solution was prepared using distilled water as solvent. The savenging activity is determined by the hydrogen donating ability. The Dilution concentration ranges from 0.6-3.0 mg/mL. There was an increase in activity as the concentration of extract increases. Thus, *B.hispida* seed has antioxidant property.

N.S.Gill*et al*.,haveperformed DDPH Radical savenging activity in *B.hispida* seed with ascorbic acid as standard drug. The extract was prepared using Methanol and the test was conducted in different concentration ranging from 100-300 µg/ml.

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Table 17. Indicates the presence or absence of activity at different concentration of extract

Concentration of extract	Results
100	+
200	+
300	+

+ Presence, - Absence

The extract showed concentration dependent savenging activity and the highest savenging activity is at concentration 300 µg/ml.

Noriham Abdullah *et al*., have performed DDPH Radical savenging activity in the pulp, seed and peel of *B.hispida*. The extracts were prepared using distilled water as solvent and standards (butylated hydroxytoluene/butylated hydroxyannisole combination and ascorbic acid) were prepared from 200ppm to 1000ppm. The savenging activity of all extracts increased from 200ppm to 600ppm but seed extract showed highest savenging and pulp extract showed the lowest activity.

ABTS Radical Cation ($ABTS^{\bullet+}$) Scavenging Activity

NadiraBinteSamad*et al*., have performed ABTS Radical Cation ($ABTS^{\bullet+}$) Scavenging Activity in *B.hispida* seed with BHT as reference. The savenging ability is measured by the ability of antioxidant molecule to quench the $ABTS^{\bullet+}$. The Dilution concentration ranges from 0.6-3.0 mg/mL. There was an increase in activity as the concentration of extract increases but the acivity of BHT sample was reduced as the concentration increases.

Hydroxyl Radical Scavenging Activity

NadiraBinteSamad*et al*., have performed Hydroxyl Radical Scavenging Activity in *B.hispida* seed with standard BHT as reference. Hydroxyl Radical Scavenging Activity is the ability of B.hispida seed extracts and BHT to savage Hydroxyl Radical. The Dilution concentration ranges from 0.6-3.0 mg/mL. The extracts with low concentration (0.6-1.2 mg/mL) showed low savenging activity but the extract concentration higher than 1.2 mg/mL showed high savenging activity similar to standard BHT. Hence, *B.hispida* seed extract can be a good source to protect oxidative cells.

Ferric Thiocyanate Method- (Nadirabinte samad et al., 2012) performed antioxidant capacity in Linoleic acid emulsion to study the inhibition of linoleic acid oxidation of aqueous *B.hispida* seed extract. BHT was used as positive control. Dilution concentration ranging from 0.6-3.0 mg/mL was oxidized for 6 days at 40°C. At initial time the inhibition percentage of was low but after 4 days the inhibition percentage was maximum and gradually decreased. The sample showed significant increase in inhibiting linoleic acid oxidation after 6 days. This shows that both the oxidation capacity of extract as well as inhibition of linoleic acid oxidation has gradually decreased with time.

β–Carotene bleaching assay

Noriham Abdullah *et al*., 2012 have performed β–Carotene bleaching assay to measure the antioxidant activity of seed, pulp and peel extracts of *B.hispida*. The standard (butylated hydroxytoluene/butylated hydroxyannisole combination) and seed extract showed inhibition of discoloration of β–Carotene but the peel and pulp extract showed significant difference.

Ferric Reducing Antioxidant Power (FRAP) Assay

Noriham Abdullah *et al*., have performed FRAP Assay to measure the antioxidant activity of seed, pulp and peel extracts of *B.hispida*. Compared to pulp and peel extract the seed extract showed highest antioxidant potential in FRAP assay. The highest antioxidant potential could be due to the presence of high phenolic contents.

Scavenging of Hydrogen Peroxide- (Rajesh kumar sharma et al., 2014) performed antioxidant activity of seed extracts of *B.hispida*. The extract was prepared using different solvent (acetone, chloroform and water). Standard ascorbic acid was used as standard drug. The Acetone extract showed more savenging whereas aqueous extract showed least savenging activity.

N.S.Gill*et al*.,have performed antioxidant activity of seed extracts of *B.hispida*. The extract was prepared using Methanol. Standard ascorbic acid was used as standard drug. The test was conducted using different concentration (25-200µg/ml).

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Table 18. Indicates the presence or absence of activity at different concentration of extract

Concentration of extract	Results
25	+
50	+
100	+
200	+

+ Presence, - Absence

The extract showed concentration dependent savenging activity and the savenging activity at concentration 200 µg/ml was comparable with ascorbic acid savenging activity.

VII. ANTICANCER ACTIVITY

Wafaa E. Soliman*et al*., have performed anticancer activity of AgNPs synthesized from the peel extract of *B.hispida* by a 96-well micro titer plate. The activity was tested on cancerous cell line HeLa and primary cell line primary Osteoblasts at varying AgNPs concentration (0.363, 0.176, 0.0922, 0.036, 0.0222, and 0.0162 µg/mL). HeLa when incubated with AgNPs with concentration ranging from 0.0116 to 0.156 µg/mL lost its viability but not significantly decreased with tested with higher concentration ranging from 0.156 - 0.313 µg/mL. The test against primary Osteoblasts showed less toxicity as cancerous cell line uptake higher AgNPs compared to primary cell line.

VIII.ANTI-INFLAMMATORY ACTIVITY

N.S.Gill*et al*.,have performed anti-inflammatory activity of seed extracts of *B.hispida* in carrageenan induced winter paw edema in wistar rat. The extract were prepared using methanol and tested at different concentration 100-300 mg/kg and measured at intervals of 60, 120 and 180, respectively. Diclofenac was used as standard and carrageenan 1% was used as disease control.

Table 19. Indicates the percentage activity at different concentration of extract

Dose	Paw reduction
100 mg/kg	19.4%
200 mg/kg	41.7%
300 mg/kg	59.7%

The extract showed dose dependent anti-inflammatory activity. Dosage 200 and 300 mg/kg showed significant reduction in paw edema compared to 100 mg/kg.

IX.ANALGESIC ACTIVITY

N.S.Gill*et al*.,have performed two tests to confirm the analgesic activity of seed extracts of *B.hispida* in mice. The extract were prepared using methanol and tested at different concentration 100-300 mg/kg and measured at intervals of 30, 60, 90 and 120 after administration of the extract. Morphine was used as standard and 1% CMC as control.

Tail immersion test- The tail of the mice was immersed in warm water. The withdrawal time was measured at regular time intervals and a cut off time was maintained to prevent skin damage. The 200 and 300 mg/kg doses showed significant difference in analgesic effect compared to control. The maximum effect was observed at 90 min interval.

Tail flick test- The tail of mice was placed on radiant heat source. The withdrawal time was measured at regular time intervals and a cut off time was maintained to prevent skin damage. The 200 and 300 mg/kg doses showed significant difference in analgesic effect compared to control. The maximum effect was observed at 90 min interval.

These tests shows that there is a significant reduction in pain sensation at medium (200 mg/kg) and (300 mg/kg) high doses.

X. CONCLUSIONS

All parts of *Benincasahispida* was found to nutritional and medically important due the presence of phytochemical compounds. Different solvents had different effect on extract. Thus, it is clear that solvent influences the effect of extract. *Benincasa hispida* is considered to be highly antibacterial against a wide range of Gram positive and

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Gram negative bacteria that are human pathogen. It also has the ability to control blood glucose level in diabetic rats and controls the viability of Human cervical cancer cell line (HeLa cell line). A wide range of Antioxidant tests has been performed to show its antioxidant activity and all tests showed positive results. It also reduces inflammation in carrageenan induced winter paw edema and also shows analgesic effect in mice. Hence, *Benincasa hispida* is found to have Antibacterial, Antifungal, Antioxidant, Anticancer, Antidiabetic, Anti-inflammatory and Analgesic activity. Compared to the pulp and peel the seed is found to be more beneficial this might be due to the presence of high phenolic content.

REFERENCES

1.Sheemole M.S, V.T. Antony, Kala K, Asha Saji., 2016., Phytochemical Analysis of *Benincasa hispida*(Thunb.)Cogn. Fruit Using LC-MS Technique., Int. J. Pharm. Sci. Rev. Res., 36(1)., 244-248.

2.Vedha Balakumar, M.Gopalakrishnan and T. Sekar., 2018., Elucidation of bioactive compounds from different polarity extracts of Benincasa hispida (Thunb) Cogn., fresh fruit pulp using GC-MS analysis., J. Global Trends Pharm Sci., 9(2)., 5322- 5330.

3.Sunayana Sharma, Hirday Narain Verma, and Nilesh Kumar Sharma., 2014., Cationic Bioactive Peptide from the Seeds of *Benincasahispida*., Hindawi Publishing Corporation International Journal of Peptides., Volume 2014.

4.Wafaa E. Soliman, Salman Khan, Syed Mohd Danish Rizvi, Afrasim Moin, Heba S. Elsewedy, Amr S. Abulila and Tamer M. Shehata., 2020., Therapeutic Applications of Biostable Silver Nanoparticles Synthesized Using Peel Extract of *Benincasa hispida*: Antibacterial and Anticancer Activities., Nanomaterials., 10.

5.D.Natarajan, R.J Lavarasan, S.Chandra babu, M.A.C.Sahib Thambi Refai, L.H.Thameemul -Ansari., 2003., Antimicrobial studies on methanol extract of *BENINCASA HISPIDA*Cogn., fruit., Ancient science of life., Vol: XXII (3) January / 2003., 98-100.

6.Rajesh Kumar Shah, Chandrika Sarmah and Sudesna Hazarika., 2015., Qualitative Analysis of Metabolites and Bioactivity Determination of Different Solvent Extracts from Seeds and Leaves of *Benincasa hispida* against Selected Microbes., Adv. Biores., Vol 6 (6) November 2015: 60-64.

7.Rekha Jadon, Shruti Dixit., 2014., Photochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains., Journal of Medicinal Plants Studies Vol. 2 Issue. 3., 58-63

8.Raju N Patil, Ravindra Y Patil , Bharati Ahirwar, Dheeraj Ahirwar., 2011., Evaluation of antidiabetic and related actions of some Indian medicinal plants in diabetic rats., Asian Pacific Journal of Tropical Medicine (2011)., 20-23.

9. Nadira Binte Samad, Trishna Debnath, Hai Lan Jin, Bo Ram Lee, Pyo Jam Park, Seung Yuan Lee and Beong Ou Lim., 2012., Antioxidant activity of *Benincasa hispida* seeds., Journal of Food Biochemistry ISSN 1745-4514.

10.Noriham Abdullah, Wan Saidatul Syida Wan Kamarudin, Zainal Samicho, Khairusy Syakirah Zulkifli and Nurain Aziman., 2012., Study on Antioxidant Capacity and Phenolic Content of Various Parts of Wax Gourd (*Benincasa hispida*)., World Applied Sciences Journal 19 (7): 1051-1056.

11.Rajesh kumar sharma, Rajni singh, K.K.Jha, Abhishek B., 2014., Antibacterial and Antioxidant activity of *Benincasa hispida* using Hydrogen peroxide scavenging model., Indian J. Pharm. Biol. Res.; 2(1):86-94.

12.N.S.Gill, K.Dhiman, J.Bhajwa, P.Sharma and S.Sood., 2010., Evaluation of free radical savenging, Anti-inflammatory and Analgesic potential of *Benincasa hispida* seed extract., International journal of pharmacology 6(5): 652-657.